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A Manual for the Use of Variable Chlorophyll Fluorescence in the Assessment of the Ecophysiology of Conifer Seedlings

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PREFACE

To achieve the greatest success with forest plantation establishment, tree seedlings must be of the highest physiological quality. An ability to assess the physiological status of tree seedlings, accurately and quickly, is crucial for determining seedling stock quality.

The integrating variable chlorophyll fluorescence (F_{var}) system provides a rapid, non-invasive and non-destructive assessment of the mechanisms by which environmental factors and nursery practices affect the photosynthetic physiology of intact seedlings. *To use this system most effectively, the user is advised to obtain a catalogue of fluorescence responses (and other physiological and morphological assessments) for individual species throughout the year, especially for critical developmental stages and cultural conditions.*

This manual provides nursery personnel, nursery managers, and researchers with information on variable chlorophyll fluorescence as an important tool for assessing seedling physiological quality. It describes the fluorometer system, instructs in its use, gives protocols for the acquisition of data, and provides assistance in the interpretation of data. The manual also explains the physiological basis of F_{var} assessment, and provides seasonal baseline examples for this physiological assessment.

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PART I

APPLICATION OF CHLOROPHYLL FLUORESCENCE ASSESSMENT

1 INTRODUCTION

Costs of seedling production and planting require that tree seedlings be of the highest possible quality—a concern to nursery manager and regeneration forester alike. "Quality" has been defined as "fitness for purpose" (Sutton 1979; see also review of Puttonen 1989) and characterization of the performance potential of seedlings has become a critical part of plantation establishment research.

Current criteria for seedling quality are based mainly on morphological characteristics. Although these are simple to use, correlations with outplant success are inconsistent and not indicative of vigour or non-visible damage of seedlings (Sutton 1982; Hobbs 1984). Root growth potential (RGP) is an example of an integrative approach to prediction of seedling performance (Burdett 1987).

Physiological characterization has become important to stock quality assessment within the last decade (Zaerr and Lavender 1980; Holbo *et al.* 1981; Zaerr 1985; Puttonen 1989; Hawkins and Binder 1991). The advantage of physiological tests is that they can indicate seedling quality in relation to nursery production, as well as measure performance potential on a particular site.

Unfortunately, most physiological tests fail at least three of the seven criteria considered to be essential to the ideal physiologically based vigour test (Zaerr 1985; Hawkins and Binder 1991). Most are not rapid and cannot be applied at all operational levels, and, because they are destructive tests, plants cannot be retested at a later time or outplanted. The variable chlorophyll fluorescence (F_{var}) assessment comes much closer to the ideal physiological test because it is non-invasive and non-destructive, and it takes only a few minutes.

The integrating fluorometer system described in this manual is designed to monitor conifer seedlings, according to defined sampling protocols, throughout the nursery production cycle. Properly used, it can provide an assessment of the physiological status of a seedling crop at any time during the growing season (Vidaver *et al.* 1989b), at the time of lifting (Vidaver *et al.* 1989a), and during or after cold, dark storage (Vidaver *et al.* 1989a).

The integrating fluorometer system can serve as a key part of a "physiological tool-kit," for use in assessing and managing seedling stock quality and performance. It is *not* intended to provide an indication of seedling vigour or quality from a single measurement in time. Replicated F_{var} measurements obtained with standardized seedling pretreatment and protocols, and forming part of a seasonal or experimental trend series, provide the best data for management and reforestation decisions.

This research methodology, currently in wide use in Scandinavia and Europe and increasing in North America, has developed into an assessment system with application at the tree seedling production and forest regeneration level. The manual has been written to accommodate two kinds of users: 1) those monitoring and assessing stock quality under current operational nursery practices; and 2) those researching basic seedling physiology to improve stock quality and nursery cultural practices.

New users of the F_{var} assessment system should become familiar with Part I, Sections 2–4 of this manual. These sections describe chlorophyll fluorescence, the Kautsky curve (F_{var} transient), the integrating fluorometer hardware, and seedling pretreatment and protocols for F_{var} measurement. Part I, Section 5 provides examples of F_{var} data on seedling responses to environmental variables and effects of nursery practices on seedling status. Part II, "Environmental and Physiological Bases of Chlorophyll Fluorescence Assessment," are discussed in more detail in Sections 6 and 7. Section 6 reviews the current understanding of the physiology and biochemistry of photosynthesis for those wishing to apply F_{var} to research in studies on tree improvement, the physiology of plant adaptations to stress, plant ecophysiology, and the genetic implications of photosynthetic mechanisms. Section 7 provides a physiological background for users wishing to extend the use of F_{var} to new applications.

2 CHLOROPHYLL FLUORESCENCE AND THE KAUTSKY CURVE

2.1 Chlorophyll Fluorescence

Of the solar radiation incident on a leaf surface (I), some is reflected (R), some is absorbed (A), and the remainder is transmitted (T), so that: $I = R + A + T$ (Fig. 2.1). The amount of light absorbed by a leaf largely depends on epidermal characteristics (pubescence and waxes), anatomical and ultrastructural characteristics of the leaf (e.g., sun or shade grown), and the pigment composition (chlorophyll and accessory pigments). Of the photosynthetically active part of incident radiation (PAR, 400–700 nm waveband), which amounts to about 45–50% of the total incident radiation (Larcher 1983; Coombs *et al.* 1985), peak absorption by chlorophylls *a* and *b* occurs in the blue and red regions of the spectrum. The leaf transmits and reflects some of the green waveband.

Energy absorbed by chloroplasts involves two pigment systems (Photosystem I [PSI] with an absorption peak near 700 nm and Photosystem II [PSII] with peak absorption near 680 nm), which are involved in the photochemical light reactions.[1] Not all of the absorbed light energy, however, is involved with photosynthesis. Some may be dissipated and lost as heat, and some may be emitted as far-red fluorescence (see Fig. 2.2 for the chlorophyll absorption, fluorescence emission, and photosynthetic light action spectra). As the photosynthetic, heat dissipation, and fluorescence emission processes are competitive (Krause and Weis 1988; Bolhar-Nordenkampf *et al.* 1989), any internal or external factor that changes the photosynthetic rate will produce corresponding changes in fluorescence emission.

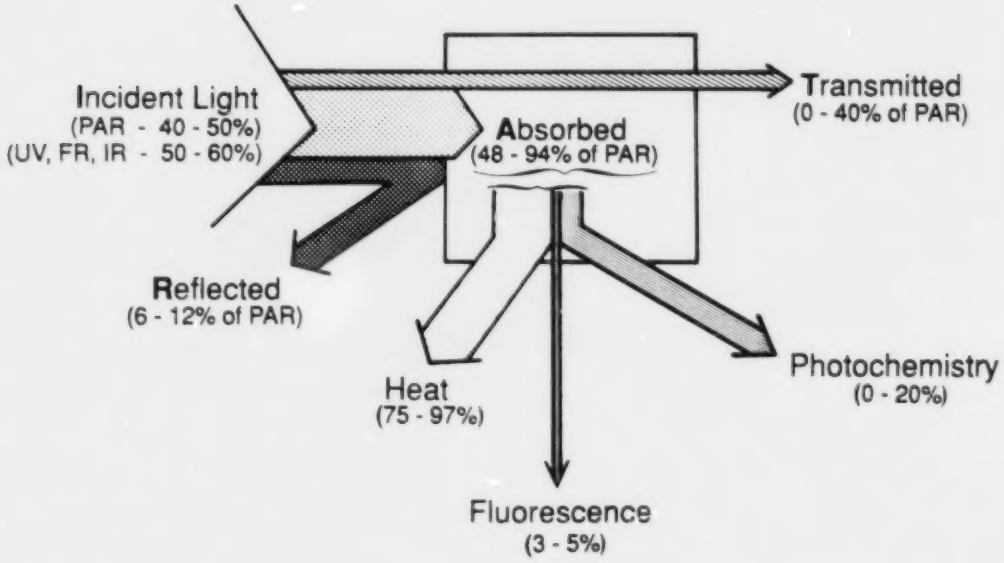


FIGURE 2.1. The disposition of light incident upon conifer foliage. The fate of absorbed radiant energy can be any combination of: 1) utilization in photochemistry; 2) de-excitation — energy re-emitted as a photon — FLUORESCENCE; and 3) de-excitation — energy dissipated as heat. (Data from various sources including: San Pietro *et al.* 1967, Larcher 1983, Nobel 1983, and Coombs *et al.* 1985.)

[1] Those not familiar with the photosynthetic system should refer to a standard text such as Hipkins and Baker 1986b, or refer to Figure 6.2 on page 42 of this manual

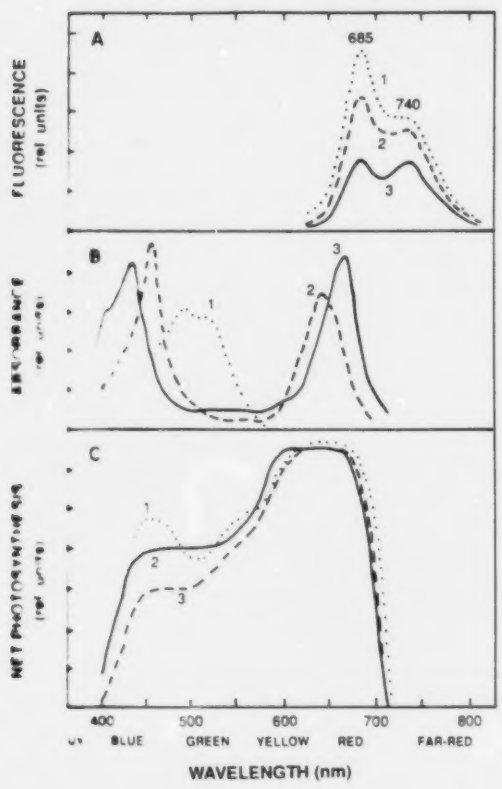


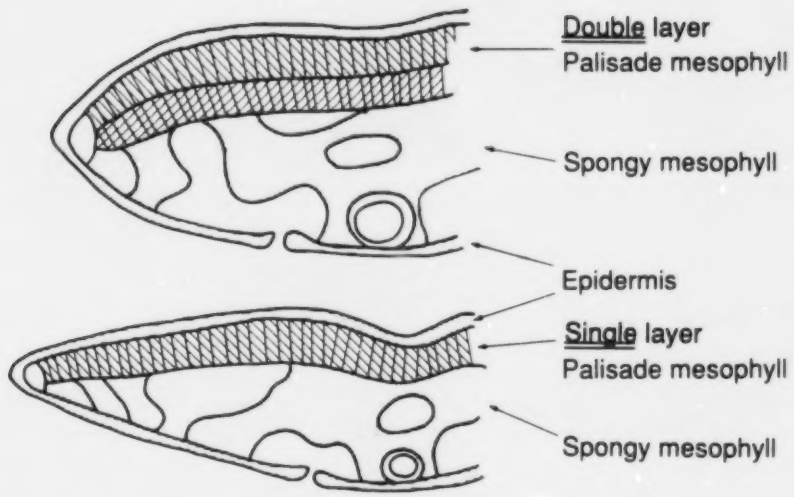
FIGURE 2.2. **[A]** Fluorescence emission spectra of European silver fir needles of different chlorophyll content: (1) Light green needles of current year; (2) Fully greened needles of current year; and (3) Dark green needles of previous year. (Adapted from Lichtenthaler *et al.* 1986). **[B]** Generic absorption spectra for (1) carotenoids, (2) chlorophyll *b*, and (3) chlorophyll *a*. **[C]** Normalized net photosynthesis light action spectra for (1) red alder, (2) coastal Douglas-fir, and (3) Sitka spruce. (Adapted from Clark and Lister 1975.)

As a result, chlorophyll fluorescence emission has become an important intrinsic probe of the photosynthetic system of plants, and therefore a powerful tool for evaluating the physiological status of a plant. It has been used to assess the functioning of the photosynthetic apparatus (Krause and Weis 1988), heat, light and drought stress effects on plant leaves (Schreiber and Bilger 1987; Lichtenthaler and Rinderle 1988), and cold acclimation, freezing stress and photoinhibition (Krause and Somersalo 1989).

During early development conifer seedlings are grown at close spacing and their architecture changes from a single shoot to a branched system. As a result, the light environment becomes increasingly heterogeneous around the leaves and seedlings soon represent a composite of sun- and shade-adapted leaves (see Fig. 2.3 and Table 2.1 for differences). In addition to time and spatial variations that influence the quantity and quality of light conifer seedlings are also exposed to variations in temperature, humidity and CO_2 concentration in the above ground environment, and to differences in water and nutrient supply in the root environment. All of these, alone or in combination, may alter photosynthetic rates and productivity.

Plant responses to these changing conditions are easily monitored with the integrating fluorometer assessment system. Unlike photosynthesis and respiration (CO_2 exchange) measurements, the determination of fresh and dry weights, chlorophyll content or leaf area is not required as a basis for interpreting fluorescence responses because the data are normalized for differences in shoot size (see Section 2.2, p. 7).

A. Sun



B. Shade

FIGURE 2.3. Sun- **(A)** and shade-grown **(B)** needle morphology in western hemlock. (Adapted from Tucker and Emmingham 1977.)

TABLE 2.1. Characteristics of leaves grown in different light environments (+ indicates a greater amount, number, or size). (Adapted from data in Coombs *et al.* 1985 and other sources.)

	Sun leaves	Shade leaves
Area (cm^2) of an average leaf		+
Ratio of palisade/spongy mesophyll	+	
Chlorophyll content (mg/unit leaf area)	+	
(mg/g dry weight of leaf)		+
Chlorophyll a/b ratio	+	
Chlorophyll/cytochrome f ratio		+
Beta-carotene content	+	
Net photosynthesis rate (mg CO_2 m^{-2} s^{-1})	+	
Dark respiration rate (mg CO_2 m^{-2} s^{-1})	+	

There are two main kinds of fluorometer systems: 1) those which use fibre optics to guide low to high excitation light intensities to a relatively small (few square millimetres) leaf surface area (see Bolhar-Nordenkampf *et al.* 1989); and 2) those enclosing an intact seedling shoot in an integrating sphere and using low to moderately high excitation light intensities (Toivonen and Vidaver 1984; Dubé and Vidaver 1990). A distinct advantage of the sphere type fluorometer is that it integrates fluorescence emission signals from an entire shoot (stem and needles). This avoids the difficult sampling problem of selecting a portion of a shoot, or several samples of a few needles from different parts of the shoot, to assess the physiological status of the seedling. Both kinds of fluorometers are capable of providing information on the fluorescence emission kinetics.[2]

2.2 The Kautsky Curve

The fluorescence emission kinetics from a dark pretreated plant that is suddenly illuminated has a characteristic response, with time referred to as the Kautsky curve (Fig. 2.4). The features of this curve are conventionally termed OIDPSMT (Papageorgiou 1975). The initial parts of the curve (OIDP) represent the fast response or phase of the fluorescence transient and are completed in about 1–2 seconds (Fig. 2.4 A). Later parts (PSMT) represent the slow response or phase of the curve and are completed in several minutes (Fig. 2.4 B). Depending on the duration of the fluorescence emission scan, the rate of emission signal acquisition, and the condition of the plant, all or only some of these fluorescence emission features may be resolved. Differences, for example in the excitation light intensity, length of the dark pretreatment, stage of foliage development, shoot age, and species, can result in modifications in the shape of the Kautsky curve. It is important, therefore, that a strict pretreatment and sampling protocol be followed (see Section 4).

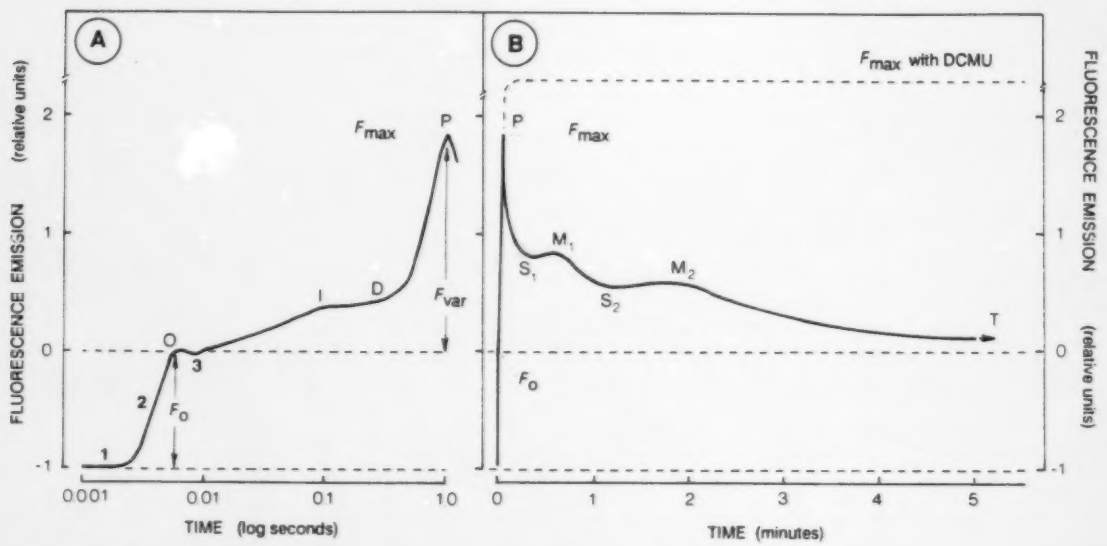


FIGURE 2.4. Form and features of a normalized fluorescence induction or Kautsky curve. **(A)** Features of the fast transient including the rapid rise to [P] (log plot after Murphy 1990). There is a lag (1) of about 3 ms from trigger signal to start of shutter opening. Full shutter opening (2) takes about 3 ms. Shutter "bounce back" or rebound may be resolved (3) at fastest rates of data acquisition. **(B)** Features of the slow transient (0–5 min time course). Both transients are typical of those for conifer needles measured at 20–23°C, excitation light intensity 95 µmol m^{-2} s^{-1} PFD following a 15–20 min dark pretreatment. Not all of the O I D P S M T Kautsky curve features may be seen in all curves and, in some cases, additional S_2 and M_2 transients may be present. See text for a complete, detailed description of the curve features.

Features of the Kautsky curve illustrated in Fig. 2.4 are outlined below:

[O]-level (= Origin)[3]: As the shutter fully opens and the dark pretreated sample is illuminated, there is a rapid rise in fluorescence emission (F). This initial level, F_o, represents the fluorescence emission when most or all of the PSII reaction centres are open (i.e., the PSII reaction centres can accept and pass on electrons when the primary acceptor, Q_A, is maximally oxidized[4] and therefore resistance to energy flow is at a minimum

[2] Fluorescence emission kinetics = time course and features of the fluorescence curve measured in relative units.
[3] Bracketed names for curve features follow Lichtenthaler and Rinderle (1988).

[= maximum photochemical quenching]). The "O-level" or F_o has been termed the initial, dead or ground fluorescence. It is derived from processes in the chlorophyll antennae pigments which are independent of any photochemical event (Lichtenthaler and Rinderle 1988). Environmental stresses that may cause structural alterations at the PSII chlorophyll pigment level can affect F_o (Krause and Weis 1984).

In addition to *initial, demo* or *ground* fluorescence, F_o has been referred to as the "constant yield fluorescence component" (Papageorgiou 1975). It is therefore a useful level against which to standardize or normalize subsequent fluorescence signals, as it accounts for differences in shoot size (i.e., F_o is proportional to the total emitting sources of chlorophyll fluorescence from the sample). As F_o is a critical measurement and used for normalization of the fluorescence transient (Fig. 2.4 A), its determination depends on a consistently resolved curve feature, or on an algorithm such as double regression, based on the rise of the initial fluorescence signal acquired at a very fast rate (Hipkins and Baker 1986a; Dubé and Vidaver 1990).

A curve feature that can be consistently resolved to yield an estimate of F_o is the maximum fluorescence signal measured on the initial rise of the transient at full shutter opening (see Fig. 2.4 A). Immediately following this maximum emission signal, a small dip in the fluorescence signal occurs (which can be seen at maximum rate of data acquistion) in response to shutter "bounce-back" and a momentary decrease in the excitation light intensity into the sphere. Thus, normalized fluorescence responses can be averaged as they are corrected for sample size differences (see Section 3, p. 13). It must be noted, however, that F_o may not always be a constant over the duration of the response curve as, for example, excitation energy transfer from the antenna to reaction centres of PSII may become impeded or the PSII reaction centres damaged (Bolhar-Nordenkampf *et al.* 1989).

The fluorescence rise above [O]-level or F_o to a maximum value (usually [P]-level, but sometimes [M]-level) represents the variable fluorescence, F_{var} (or F_v), which only occurs in photosynthetically active plant tissue. Fluorescence increases from [O] to [P] as the Q_A pool becomes increasingly reduced and the water-splitting part of the photochemistry overtakes electron transport.

Curve features of the fast response from [O] to [P]-level are (Fig. 2.4 A):

- [*I*]-*level* (= *Intermediate*): The rise from F_o to [I] represents rapid reduction of Q_A associated with water-splitting system charge accumulation.
- [*D*]-*level* (= *Dip*): The [I to D] transient may be a dip or plateau, if apparent, on the induction curve. It indicates the oxidation of Q_A as Q_B is reduced by electron transfer from Q_A.
- [*P*]-*level* (= *Peak*): At this level, Q_A and the plastiquinone pool (see Fig. 6.2) are highly reduced and the photochemical quenching of fluorescence approaches zero (Hipkins and Baker 1986a). Maximal closure of the reaction centres or traps to the excitation energy is believed to occur at this level (Bolhar-Nordenkampf *et al.* 1989). Often [P] is given as the maximum fluorescence yield (F_{max}). However, this fluorescence yield may not reach the maximum reduction of Q_A (and consequently maximum fluorescence level) known to occur in the presence of the photosynthetic herbicide DCMU, which blocks the transfer of electrons from Q_A to Q_B (Fig. 2.4 B).

Some interpretations of the Kautsky curve are based on ratios of the variable component of fluorescence ($F_{max} - F_o = F_{var}$ [or F_v of some authors]) and [P]-level (F_p) or F_{max} level (Fig. 2.4 A). Assumptions and potential sources of error inherent in this ratio are discussed in Bolhar-Nordenkampf *et al.* (1989).

The length of the dark pretreatment time (Ireland *et al.* 1984), the level of the excitation light intensity (Krause and Weis 1984), and the relative stages of leaf development in a shoot sample (see Section 5, Fig. 5.6) can all influence the height of [P].

Interpretation of the slow response or quenching phase of the fluorescence transient [PSMT] is complex (Fig. 2.4 B). More than one [S] and [M] feature may occur during this slow phase, resulting in curve designations of [S_1, M_1, S_2, and M_2]. From the [P]-level, a decrease in fluorescence (i.e., quenching, see Fig. 2.4 B) normally occurs as photochemistry increases in response to electron transfer away from Q_A. The

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[4] *Oxidation* = loss or removal of electrons; *reduction* = gain or addition of electrons.

[*S*]-*level* (= *first Steady state*) and [*M*]-*level* (= *Maximum or second maximum*) (Lichtenthaler and Rinderle 1988) are associated with the induction of net photosynthetic CO_2 assimilation (Fig. 2.5) (Ireland *et al.* 1984; Hipkins and Baker 1986a). The fluorescence and CO_2 fixation curves then appear to follow an anti-parallel pattern until steady state conditions are reached, for example in *Zea mays* shown in Fig. 2.5 (Ireland *et al.* 1984).

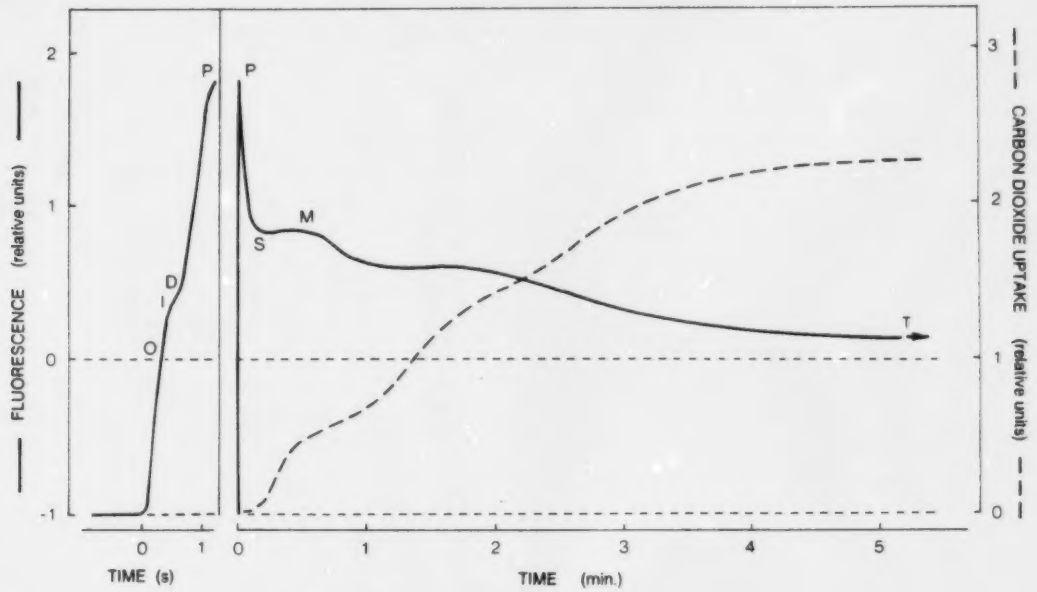


FIGURE 2.5. Time courses for CO_2 uptake rate and fluorescence emission to steady state conditions for dark pretreated maize leaves on sudden illumination. Note that the sudden initial rise in CO_2 uptake coincides with the [S] to [M] transients following [P]. Steady state conditions may require more than 5 min. (Adapted from Hipkins and Baker 1986a.)

The [*T*]-*level* (= *Terminal state*) represents the steady state phase of the fluorescence transient. Quenching of the fluorescence transient between [P] and [T] is the result of a balance between two types of quenching: photochemical and non-photochemical. Non-photochemical quenching (decreased fluorescence emission) includes energy de-excitation processes which are non-radiative, such as heat transfer or energy transfer to PSI. Photochemical quenching (decreased fluorescence emission) is caused by energy transformations at the PSII reaction centres (Bolhar-Nordenkampf *et al.* 1989).

It is important to note (Fig. 2.5) that induction of net CO_2 fixation occurs in time at the [S - M] part of the fluorescence transient after [P], and that steady state conditions in fluorescence emission and CO_2 fixation are not reached for several minutes after sudden illumination of a dark pretreated sample.

3 THE INTEGRATING FLUOROMETER ASSESSMENT SYSTEM

3.1 Instrument Description

The integrating fluorometer system consists of the following components (Fig. 3.1):

- an excitation light source, heat filter and cooling fan
- an electronic shutter and glass filter train
- an integrating sphere and diffusing cone
- a photodetector and amplifier to measure fluorescence emission
- an incident light/absorbance sensor

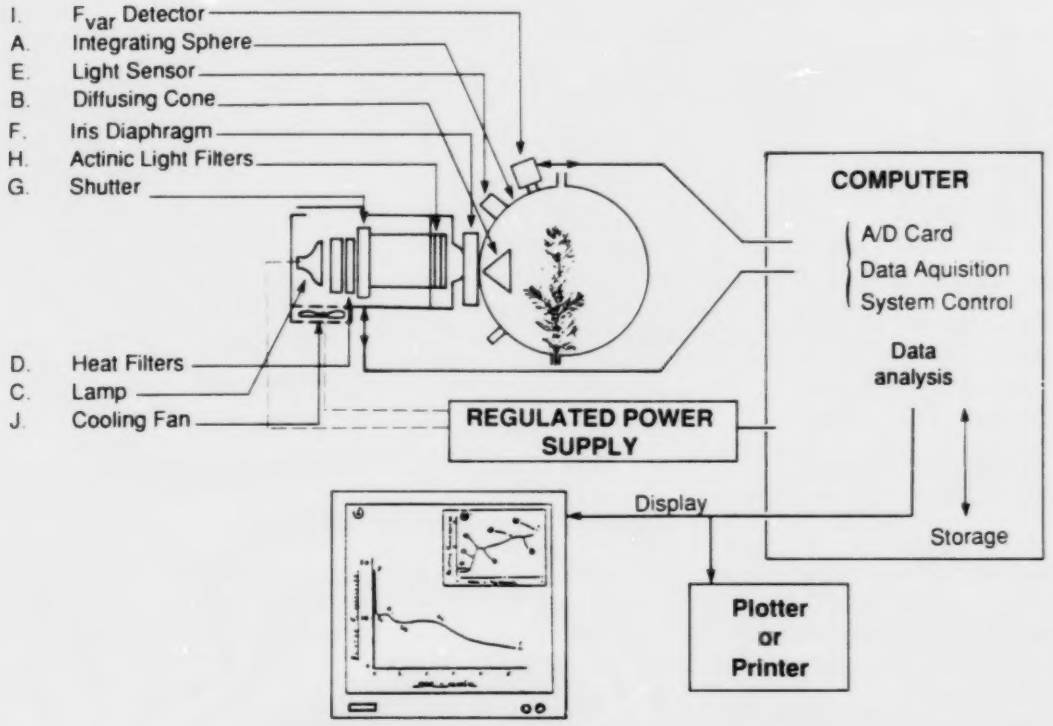


FIGURE 3.1. Components of the integrating fluorometer system. See text for detailed description.

These components are arranged in an appropriate housing and interfaced to a microcomputer with hard drive, which triggers the shutter opening and acquires and stores the fluorescence emission data at onset of the shutter opening. Important features of the main components are described below.

The 10–25 cm diameter **integrating sphere** (Fig. 3.1 A) consists of two hemispheres separable in a vertical plane at 90° from the excitation light entry port. One of the hemispheres is fitted and fixed to the excitation light train and shutter housing. Ports in the surface of this hemisphere accommodate the excitation light beam, the fluorescence detector, a photodetector to measure the excitation light, and inlet and outlet fittings to provide for atmospheric gas flow through the sphere.

Inside surfaces of the hemispheres are coated with a highly reflective material to maximize uniformity of diffuse light distribution to the sample plant. A **light diffusing cone** (Fig. 3.1 B) located inside the sphere at the excitation light port ensures that light scatters and that no direct excitation light reaches the plant material.

For measurement of chlorophyll fluorescence, the stem of the intact plant to be sampled fits into a slightly recessed opening at the base of the sphere. A closed-cell foam gasket fitted to the flanges of the hemisphere provides a light- and air-tight seal when the hemispheres are closed. Different sized spheres may be used to accommodate plants of different size and architecture.

The excitation light is provided by a broad band (wide spectrum) tungsten or tungsten-halogen **light source** (Fig. 3.1 C) fitted in a parabolic reflector and powered by a stabilized DC power supply. Care must be taken to ensure that the lamp is correctly mounted and aligned in the fixed mount. As the light source produces considerable heat it must be well ventilated and provided with a **cooling fan** (Fig. 3.1 J). A **heat absorber/heat reflector filter combination** (Fig. 3.1 D) removes excess heat from actinic light conditioning filters in the excitation beam.

The excitation light intensity or photon flux density (PFD), into the sphere should be at least one-third to one-half the photosynthetic light saturation level for the sample plant for most measurement purposes (see Fig. 3.2). This means that the lamp source should provide a photon flux density into the integrating sphere of at least 100–200 μmols m^{-2} s^{-1} PFD measured by a **photodetector** (Fig. 3.1 E) such as a LiCOR PAR sensor located on the hemisphere surface. Although voltage control can be used to vary the PFD by changing voltage supply to the lamp, this will shift composition of the spectral output from the lamp. An **iris diaphragm** (Fig. 3.1 F) can be placed in the excitation light beam to alter light intensity into the sphere if this is required.

An **electronic shutter** (Fig. 3.1 G) controls entry of the excitation light into the integrating sphere. A shutter control device provides the required voltage to open and close the shutter at computer-selected intervals. Together the shutter and its control device provide the trigger signal to control the time of excitation and duration of a measurement and this starts and stops measurement of the chlorophyll fluorescence emission from the plant. The shutter must have a rapid rise time to open fully (3 milliseconds [ms] or less) because important features of the fluorescence emission transient occur within the first few milliseconds of the dark to light transition of the sample plant when the shutter opens. **Glass filters** (Fig. 3.1 H), located between the shutter and sphere, isolate light from the lamp to an appropriate excitation wave band (less than 650 nm, see Fig. 2.2) which does not significantly overlap the fluorescence emission wavelengths (more than 650 nm).

Chlorophyll fluorescence emission from the plant has a peak wavelength of approximately 680–685 nm with a secondary shoulder at about 740 nm (see Fig. 2.2 A). The **fluorescence emission detector** (Fig. 3.1 I) is protected from the excitation wave band by appropriate glass filters which only transmit fluorescence emission from the plant. The signal from the detector is proportional to the fluorescence emission amplitude. Because energy emitted by fluorescence from excited chlorophyll of the plant is relatively weak compared to the absorbed excitation light intensity (3–5 % [Walker 1985]), and because some light (stray light) may leak into the sphere the background light (sphere without plant) must be measured before fluorescence is measured. As the amplifier associated with the fluorescence emission detector is battery powered and has a small positive voltage bias (called the dark offset), this may be noted as a battery status check and is corrected for as part of the background signal.

A **PC-XT** or **AT computer** is used to control the operation of the electronic shutter and the fluorescence measurement, and for data acquisition from the integrated detector/amplifier, data processing, storage, and analysis. An analog to digital (A/D) board installed in the computer must convert analog signals from the fluorescence detector to digital form at a rate sufficient to isolate and detect critical features of the fast and slow fluorescence transients for disk storage.

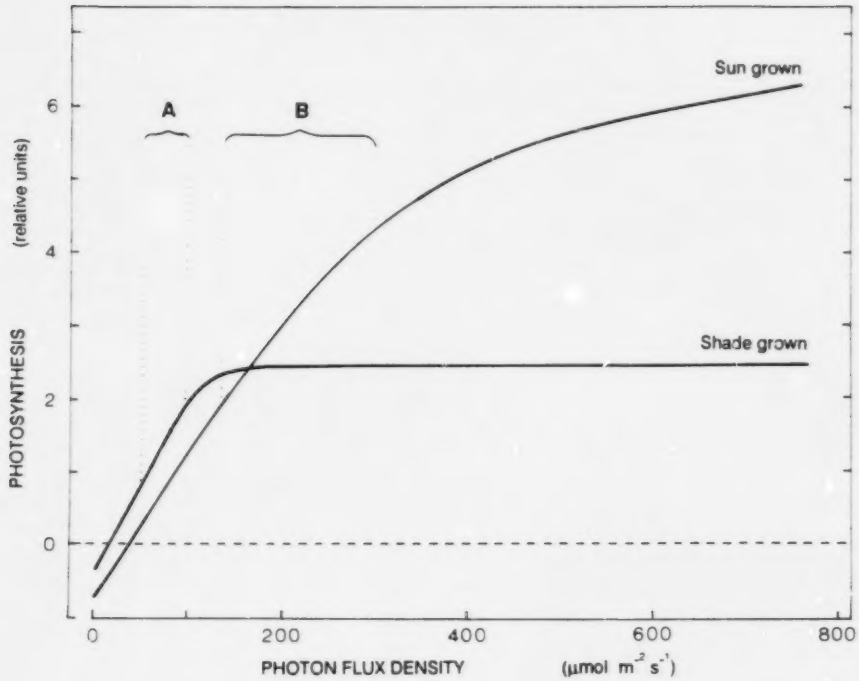


FIGURE 3.2. A generic diagram of the rate of photosynthesis at different photon flux densities for sun- and shade-grown needles. The minimum range of excitation light intensities suitable for fluorescence measurements of shade- **(A)** and sun-grown **(B)** needles is indicated. The slopes of these curves reflect the anatomical, morphological, and physiological differences of leaves given in Table 2.1.

3.2 Data Acquisition, Processing and Analysis

Software associated with the integrating fluorometer assessment system has control and data acquisition and processing functions.

The software triggers the electronic shutter to control the initiation and duration of the excitation light entry into the sphere and the resulting chlorophyll fluorescence emission signal from the plant. During the measurement, the excitation light intensity in the sphere is continuously monitored to ensure standardization and regulation of the excitation light intensity because fluorescence emission yielding F_o is directly proportional to it (Lavorel and Etienne 1977).

Once the fluorescence time response signal has been corrected for background light and the dark offset, the software determines the normalized fluorescence transient according to the following relationship:

$F_{var(t)} = [F_{v(t)} - F_o] / F_o$ where:

$F_{var(t)}$ is the normalized variable fluorescence at time t,

$F_{v(t)}$ is the corrected non-normalized fluorescence signal at time t, and

F_o is the initial fluorescence emission signal before the onset of any photochemistry.

As the F_{var} response curves are normalized to F_o, statistical treatment of the data (means and standard errors) can be determined for particular critical features of the transient (i.e., P, M, T).

The software may also be designed to provide for: random access to any fluorescence emission curve in a data file; selection of curves to be averaged; elimination of a curve from the data bank if a protocol error or equipment malfunction occurs; and both time course display on the monitor and hard copy production on either an X-Y plotter or a high quality printer.

4 SEEDLING PRETREATMENT AND PROTOCOL FOR THE F_{var} ASSESSMENT SYSTEM

Seedlings should be monitored at appropriate intervals from shortly after germination until the time of lifting. This way, the effects of watering and fertilizer regimes and other operational practices, such as blackout, CO_2 enrichment, supplemental lighting, and drought stressing, can be evaluated (Duryea 1985). Effects of natural environmental stresses can be evaluated and the lifting window delineated.

Because seedlings are usually moved from an outside growing area or environmental chamber to the laboratory for physiological assessment, the F_{var} and CO_2 exchange measurements reflect the response or physiological capacity of the seedlings *to a given set of test laboratory conditions at the time of measurement*. These test conditions, especially temperature, humidity and light, may differ notably from the environmental conditions under which the seedlings were previously held. For example, the physiological measurements of seedlings brought in from an outside growing area with near freezing temperatures will express the laboratory test response and recovery of the seedlings from their earlier low temperature exposure.

The conditions under which Quality Control Laboratory (QCL) measurements are made should be kept as constant and reproducible as possible throughout an experimental series (over days, weeks or a complete production cycle).

4.1 Seedling Pretreatment

4.1.1 Dark pretreatment

To observe a fully developed Kautsky F_{var} transient curve requires first placing the seedling in complete darkness. The time in darkness must be of sufficient duration that most, if not all, of the electron carriers are in the oxidized state and the levels of proton gradient—and hence ATP formation—are minimal. The time required for a plant to reach such a dark steady state depends on the particular plant (species and ecotype) being assayed, its stage of development, and its previous light environment, water and nutritional status. In general, a dark pretreatment period of 20–25 min has been found to be adequate. A shorter time may be satisfactory if the previous light regime was of short duration and low irradiance level. Longer dark times may be necessary (>1 h) if the irradiance levels were high (i.e., in the order of saturating intensities and above). Dark pretreated seedlings must be transferred in darkness (window shades drawn, room lights off) from the dark box or cabinet to the integrating sphere. While absolute darkness is not required, the operator should be working under "night vision adapted" conditions. A low intensity green photographic safelight may help.

4.1.2 Tissue/sphere volume relationships

There is considerable latitude in the amount or size of tissue sample that can be tested in a given sphere size. The downward limit is imposed by the ability of the system to detect significant levels of fluorescence emission. Satisfactory data have been obtained with 1–3 cm^2 of tissue-culture leaf material in an agar slant culture tube placed into the integrating sphere. Testing larger seedlings is more complex. While shoots of spruce and Douglas-fir seedlings equal in height to the sphere diameter have been successfully assayed, the gross morphology must be kept in mind. Shoots with multiple lateral branches or high needle density per unit stem length will exceed acceptable amounts of tissue even if the leader stem length is only two-thirds that of the sphere diameter. Within any one experimental series, the amount of tissue being compared should be appproximately the same. The remaining differences in size are accounted for during normalization of the data (F_o = amount of excited chlorophyll). The F_o has been

correlated to both tissue volume (see Fig. 6; Toivonen and Vidaver 1984) and needle dry weight (Murphy and Lister, 1990. Department of Biological Sciences, SFU, unpublished data). The upper limit of tissue/sphere volume relationship is imposed by the tissue itself when it becomes significantly self-shaded.

4.1.3 Aeration

Reduced air circulation can be a problem in a darkened box, cabinet or room unless special precautions are taken to duct in and circulate fresh air. Carbon dioxide concentrations can easily and quickly rise well above the average ambient level of 350 $\mu l\ l^{-1}$, and this can lead to changes in stomatal conductance during dark pretreatment and subsequent assay. Within the dark box or cabinet, the seedlings themselves quickly raise the CO_2 level via dark respiration CO_2 evolution. Also, operators breathing over the plants while handling and placing the shoot into the sphere may elevate the CO_2 concentration by 10–100 times (i.e., 0.035–3.5% or more) in expired air. Within the integrating sphere, the CO_2 concentration may be *reduced* significantly during a 5-min F_{var} determination by active CO_2 uptake. It may approach the CO_2 compensation point (no net CO_2 uptake because photorespiration equals photosynthesis) if sufficient very active tissue is present. This could lead to changes in [T] levels. An air flow should therefore be arranged within the room, dark box and sphere whenever possible, to assure normal CO_2 and humidity levels are maintained.

The following factors are also known to affect the F_{var} curve features in both time and magnitude.

4.1.4 Time of day

While preliminary studies have shown that diurnal changes in F_{var} curve magnitude and form do occur (Fig. 4.1), the significance of such changes is reduced if all measurements are carried out over the same restricted time period each day. It has become standard practice to carry out routine F_{var} assays only between ±3 h of local solar noon. The general practice is to determine F_{var} between dawn and solar noon, and then, after allowing a photosynthetic induction period, to determine the gas exchange as well as any destructive measurements such as leaf, shoot, and root water potential, dry weight, mitotic index, and chlorophyll content sampling.

4.1.5 Water regime

Care should be taken to keep the water status of the seedling as near "normal" or as close to the predetermined experimental conditions as possible during the whole assay period. Rates of water loss may increase in a room or laboratory with low relative humidity, so the experimental humidity level should be maintained and some additional water added to compensate.

4.1.6 Humidity

Relative humidity around seedlings should be maintained at 65–90% in the laboratory. Many buildings may have humidity levels well below this range, for example, during the winter months and in the summer months when temperatures rise significantly above 25°C. Before light pretreatment, clear plastic bags may be placed **loosely** over each seedling to reduce changes in water stress levels and vapour pressure deficits even in growth chambers where relative humidity is controlled.

4.1.7 Light

Seedlings transferred from their growing site to the QCL should be assayed as soon as possible. They may, however, be collected a little ahead of time before assay. In this case, they should be placed under supplementary lighting of at least 350–450 $\mu mol\ m^{-2}\ s^{-1}$ PFD for at least 30 min. Lights without heat filtering, especially incandescent sources, should be avoided.

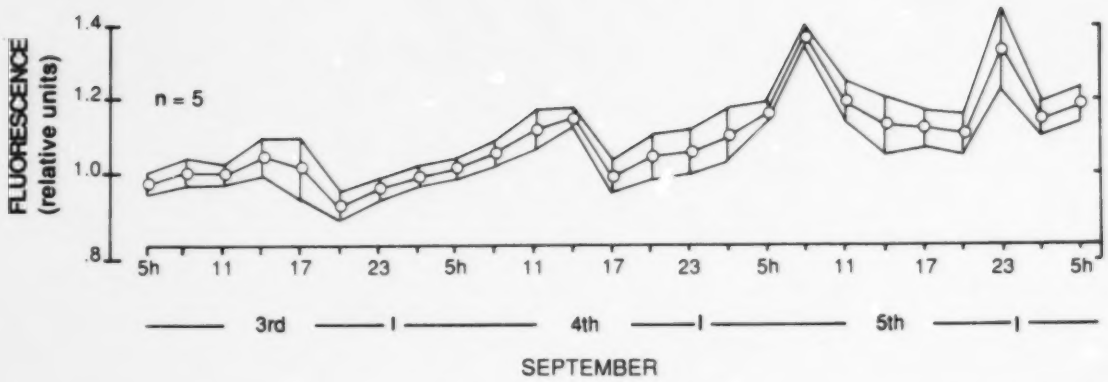


FIGURE 4.1. The diurnal fluorescence emission pattern in normalized [P] for white spruce seedlings measured over a 72-h period. Seedlings were randomly selected from plants kept in the dark at approximately 25°C in a ventilated incubator at each 3-h measurement interval. Except when the sample seedlings were exposed to 5 min of excitation light during the F_{var} assay, they were kept in the darkened incubator. Means "o" and standard error bars are shown.

4.1.8 Temperature

The preferred working range for laboratory physiological measurements is about 20–25°C, which is approximately the average yearly room temperature. The effects of local temperatures outside of this range and of significant temperature fluctuations should be ascertained.

4.2 Measurement Protocol

The general procedures used by the Forest Ecophysiology Research Group at Simon Fraser University are described below. Some of these steps may be incorporated into the system control software and modified at the operator's discretion.

1. Select sample seedlings and place them under temporary holding conditions before the F_{var} assay. These holding conditions—length of time, light, humidity and temperature—should be standardized.
2. "Turn" on the air flow systems in the dark room and the sphere (see Section 4.1.3).
3. Power up the AC/DC lamp power supply. Check that the lamp fan system is operating.
4. Activate the lamp output monitoring and recording system.
5. "Turn" the computer ON.
6. Load the control and data acquisition programs.
7. Check the shutter operation over one open/closed cycle.
8. Apply power to the integrating fluorometer lamp.
9. "Open" shutter, allow approximately 20 min (Fig. 4.2) for the lamp to achieve steady state temperature and light output conditions as indicated by the monitoring system. (Note: An open shutter will prevent heat build-up during lamp warm-up.)

10. Place the first seedling sample into the dark pretreatment box, adding the remainder in intervals such that each will have the required minimum 20–25 min dark pretreatment before assay. *Note: Do not forget to allow time for the actual assay of the previous sample (5 s to 5 min), time for handling, and time for data processing (see steps 17 and 18 below).
11. Check for lamp stabilization (approximately 20 min elapsed time) and make a final adjustment of the lamp intensity level.
12. "Turn" the detector and amplifier ON.
13. With both the sphere and shutter closed, determine, record and, if required, adjust the dark current offset of the detector.
14. Determine the stray light signal level. (This baseline correction factor will include the dark offset current factor—Section 3.1.) *Note: All extraneous lights should be OFF.
15. Make a final check that the *shutter is closed*, the lamp is on and steady, and all other lights are out.
16. Transfer a seedling to the sphere *IN THE DARK* (See Sec. 4.1.1).
17. Activate the variable fluorescence data acquisition program for the selected time course (e.g., 5 s to 5 min).
18. Display the F_{var} transient curve(s) on the computer screen.
19. Delete or save the F_{var} curve.
20. Repeat steps 16–19 until all sample seedlings have been assayed in correct time sequence.
21. Repeat step 14, compare and average the "stray light" baseline correction factor.
22. Generate and display the average normalized F_{var} curves.
23. Print or plot out the desired set of F_{var} curves.
24. Back up data on floppy discs or transfer to an alternate system.
25. Shut down all battery-powered systems ("power down").

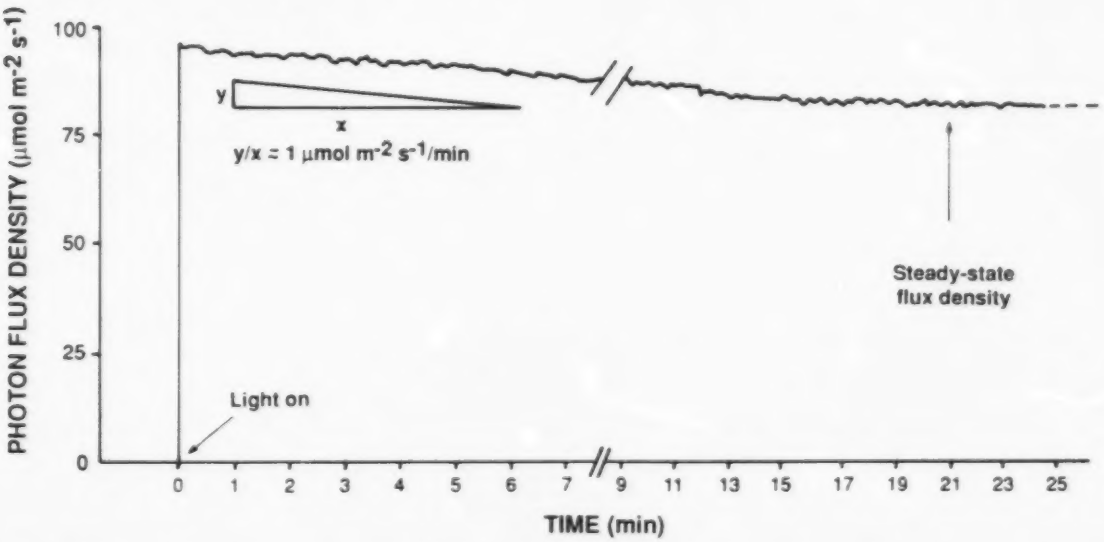
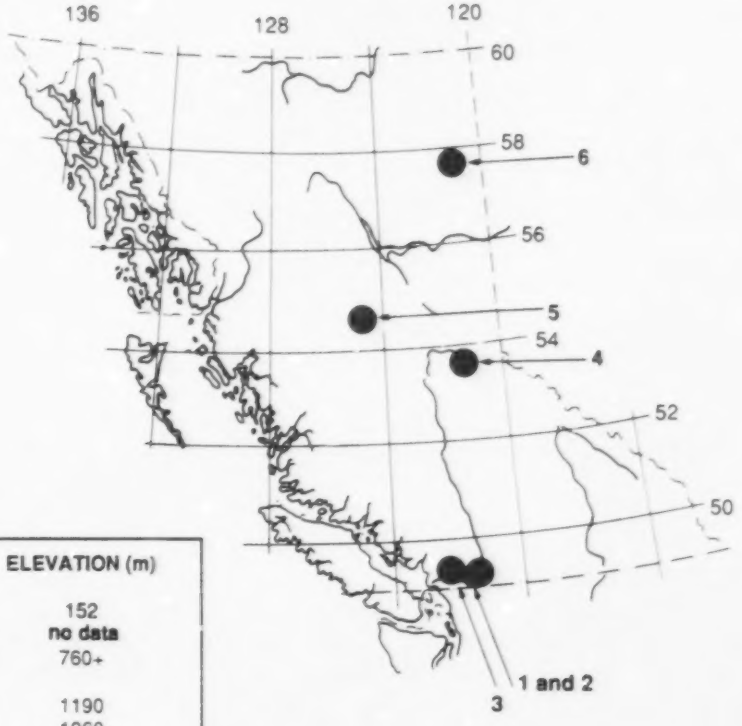


FIGURE 4.2. An example of the change in emission of the excitation lamp source during its first 25 min of operation. The rate of decline in intensity (1 μmol m^{-2} s^{-1} PFD/min) is typical of such incandescent light sources each time they are turned on.

As further research is conducted and the operator gains experience, the detailed basic protocol can be modified. The actual sequence, timing, and environmental conditions preceding and during the assay will depend on the objectives of the user and the plant material being assayed.

5 INTERPRETATION OF F_{var} RESPONSES

The following sections provide F_{var} responses of conifer seedlings obtained by the Forest Ecophysiology Research Group under laboratory conditions at Simon Fraser University. The locations of seedlots mentioned in the text are given in Figure 5.1; daylengths for selected latitudes in British Columbia are provided in Table 5.1. Responses of the conifer seedlings are arbitrarily grouped into three categories: baseline studies, long-term responses, and short-term responses. Overlap occurs in the first two categories. Baseline studies of conifer seedling F_{var} responses provide seasonal growth cycle patterns for tree species. However, it must be recognized that occasional in-season F_{var} responses reflect short-term environmental cycles—unusual cold, high temperatures, which may stand out from the general F_{var} response trend. Long-term responses are generally those cued by daylength and associated temperature change. They may take place over weeks or months. Short-term responses are cued by shorter cyclic changes in environmental conditions which, if no damage to seedlings occur, persist only as long as the changed environmental condition remains. A standard format giving species, seedlot, nursery, growing conditions, test site, and duration is provided for the examples which follow.



		SEEDLOT	ELEVATION (m)
Douglas-fir	1	1273	152
	2	4407	no data
	3	6399	760+
White spruce	4	4073	1190
	5	8534	1060
	6	8981	750

FIGURE 5.1. Location and elevation of seedlots referred to in Section 5.

TABLE 5.1. Daylength (h and min) at different latitudes in British Columbia

Month[a]	Latitude North					
	50°	52°	54°	56°	58°	60°
January	8 30	8 12	7 54	7 29	7 03	6 38
February	10 05	9 56	9 47	9 35	9 22	9 10
March	11 49	11 48	11 47	11 45	11 43	11 42
April	13 40	13 38	14 05	14 16	14 26	14 37
May	15 21	15 37	15 54	16 17	16 39	17 02
June	16 21	16 44	17 07	17 41	18 15	18 49
July	15 57	16 22	16 48	17 14	17 40	18 06
August	14 34	14 46	14 58	15 18	15 38	15 48
September	12 40	12 43	12 46	12 49	12 53	12 57
October	10 49	10 43	10 38	10 30	10 23	10 15
November	9 08	8 58	8 38	8 19	7 59	7 40
December	8 06	7 46	7 25	6 55	6 25	5 55

[a] Daylengths are for mid-month

(Source: Royal Astronomical Society of Canada 1987)

5.1 Baseline Studies

5.1.1 Seasonal fluorescence patterns of selected tree species

Douglas-fir (1+0)

Seedlots: 1273, origin 47°17'N, 122°29'W (152 m asl); 6399, Dewdney, B.C., seed orchard (760+ m asl)

Nursery, growing conditions: B.C. Ministry of Forests, Surrey, B.C. (49°N 123°W), container grown (PSB-313)

Test site: Seedlings tested at Simon Fraser University, Burnaby, B.C. (49°N 123°W; 365 m asl) within 24 h of transport from the nursery

Duration of tests: August 1988–March 1989

Results and interpretation: Late August F_{var} curves of both low elevation (1273) and high elevation (6399) Douglas-fir seedlots are similar and show some indication of water stress associated with the high temperatures at that time (Fig. 5.2). October curves show better activity with higher [P] transients and [M] and [T] levels. Late October and November 25 responses show some inactivation, with more in the high elevation seedlot (late October). January 9 curves display the most inactivated response of the curve series—the low elevation seedlot (1273) more so than the high elevation seedlot (6399). The [P] and [M] levels show some recovery in February, with a further increase in March.

The F_{var} time courses show that coastal Douglas-fir do not appear to follow the same progressive inactivation in fall as does white spruce (see Fig. 5.4), but responds to the weather conditions prior to measurement.

White spruce (1+0)

Seedlot: 8534, origin 54°N 124°W (1060 m asl)

Nursery, growing conditions: Pelton Reforestation Ltd., Maple Ridge, B.C. (49°N 123°W; 5–10 m asl); container-grown (PSB 313)

Test site: Seedlings were tested at Simon Fraser University, Burnaby, B.C. (49°N 123°W; 365 m asl) within 24 h of transport from the nursery

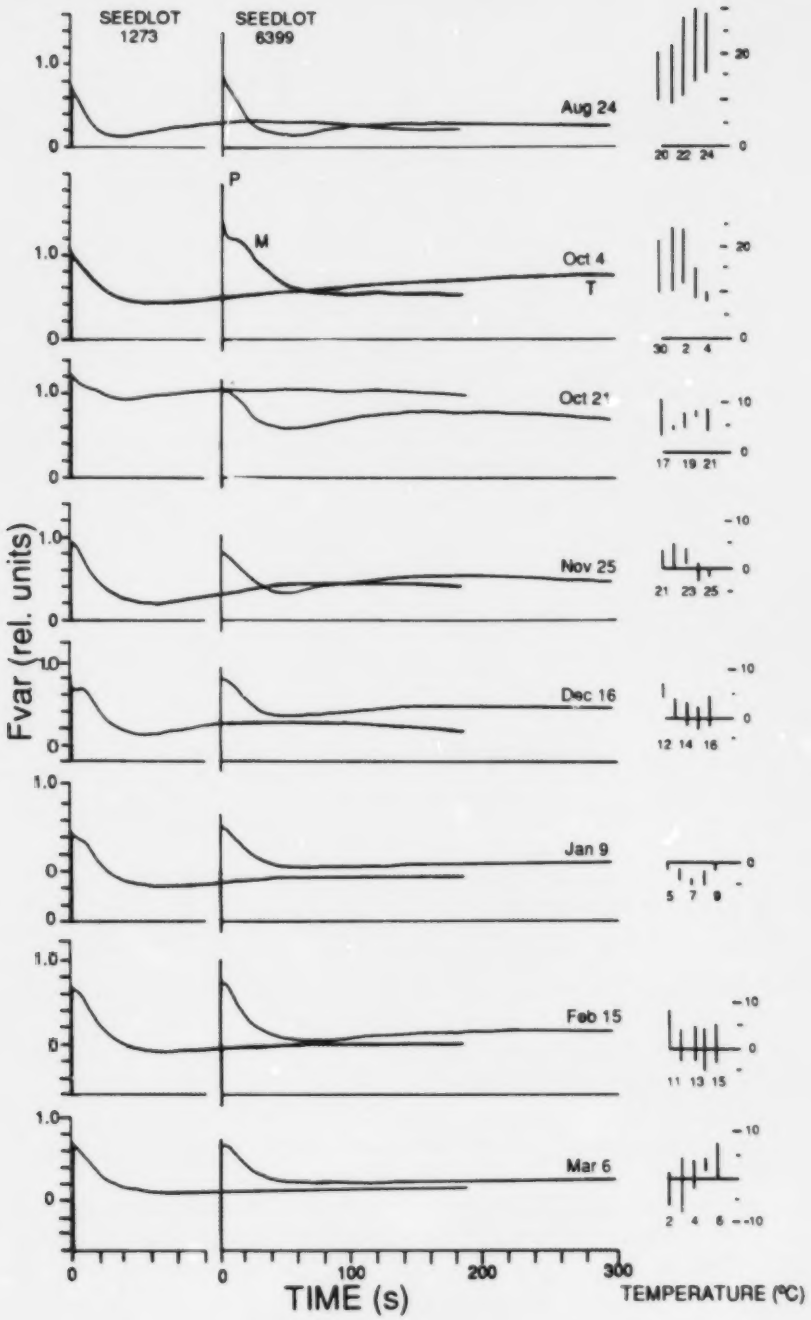


FIGURE 5.2. Mean F_{var} time courses (n=5) for low elevation (1273) and high elevation (6399) provenances of coastal Douglas-fir from August 1988 to March 1989. Daily temperature patterns for 5 days ending on the day of F_{var} measurement are given for each curve period. Note that the January 9 curves display the most inactive response in the curve series—low elevation seedlings (1273) more so than seedlings of the higher elevation provenance. The F_{var} time courses for coastal Douglas-fir suggest an opportunistic response to weather conditions preceding the measurement. (Data from Brooke *et al.* [1989] and Vidaver *et al.* [1989b].)

Duration of tests: Sept. 15, 1989–March 30, 1990

Results and interpretation: F_{var} declined gradually from mid-September until mid-November 1989 (Fig. 5.3), paralleling the decline in photoperiod and only a moderate decrease in ambient temperatures. There was a large drop in F_{var} between November 10 and 24. This corresponded with a period during which a relatively large decrease in season "normal" levels of ambient air temperatures occurred. The F_{var} [P] levels continued to decrease only gradually from November until about late December; the [T] levels dropped considerably during this period. The F_{var} remained low from late December until the beginning of March, increasing slightly by March 16. Ambient air temperatures were frequently low (usually less than 5°C; night temperatures often -1 or -2°C) from mid-January to mid-February, and well below freezing for several days before the February 19 test. Ambient air temperatures increased substantially from March 2 to 16, and there were several sunny days on which the maximum temperatures exceeded 10°C. Some swelling of buds was observed by mid-March. The seasonal pattern in APS rates (Fig. 5.3) generally paralleled the pattern observed for the F_{var} responses, although most plants were capable of positive APS rates *at room temperature* on all test dates except February 19.

Photochemical activity in white spruce is sensitive to seasonal changes in photoperiod (Vidaver *et al.* 1989b) and to ambient weather conditions. While continuing mild temperatures into the late fall slowed down the attainment of winter inactivation, a sudden drop in temperatures in late November hastened the decline in overall F_{var}. Daytime temperatures at or below freezing further suppressed F_{var} (Figs. 5.7 and 5.8). The general seasonal changes in F_{var} may parallel changes in dormancy and frost hardiness status in white spruce seedlings.

White spruce (2+0)

Seedlot: 8981, origin 58°N 120°W (750 m asl)

Nursery, growing conditions: B.C. Ministry of Forests, Surrey, B.C. (49°N 123°W), container grown (PSB-313)

Test site: Seedlings were tested at Simon Fraser University, Burnaby, B.C. (49°N 123°W; 365 m asl) within 24 h of transport from the nursery

Duration of tests: June 1987–December 1987

Results and interpretation: Starting around mid-August, the F_{var} response began to decline (Fig. 5.4). By late October or early November, F_{var} reached a low level at which time apparent photosynthetic rates were approaching zero. Seedlings were found to be cold hardy to -18°C (B.C. Ministry of Forests tests) on October 20, 1987, and were lifted for cold, dark storage (-2°C) on December 2, 1987.

White spruce exhibits daylength-dependent induction of photochemical activity reduction in the fall as cold hardiness is increased.

5.1.2 Changes in fluorescence patterns which follow bud break and flushing of new foliage

White spruce (1+0)

Seedlot: 8981, origin 58°N 120°W (750 m asl)

Nursery, growing condition: Pelton Reforestation Ltd., Maple Ridge, B.C. (49°N 123°W; 5–10 m asl), container grown (PSB-313B)

Test site: Seedlings were tested at Simon Fraser University, Burnaby, B.C. (49°N 123°W; 365 m asl) within 24 h of transport from the nursery

Duration of tests: January 1990

Results and interpretation: The photosynthetic activity of white spruce seedlings which had been cold, dark-stored (-2°C) for 54 days recovered within 2–4 days after placement in a growth chamber environment (Fig. 5.5). Pre-bud flush F_{var} curves generally show a progressive increase in the height

of F_{var} maxima as well as of [T] (F_{var} steady state), which are associated with increases in the APS rate (Fig. 5.5). A sharp [P] spike and decreased APS rates indicate the onset of flushing activity at 9 days in the growth chamber. At 17 days, new foliage was emerging from the terminal bud (flushing stage 3), APS rates were increasing and, as the new shoots elongated, a sharp [P] spike appeared again in the F_{var} curve.

A pronounced [P] spike which precedes spring bud-flushing activity is often associated with sharply declining APS rates. This indicates that the photochemical capacity of the seedling's photosynthetic system exceeds its biochemical CO_2 fixation capacity.

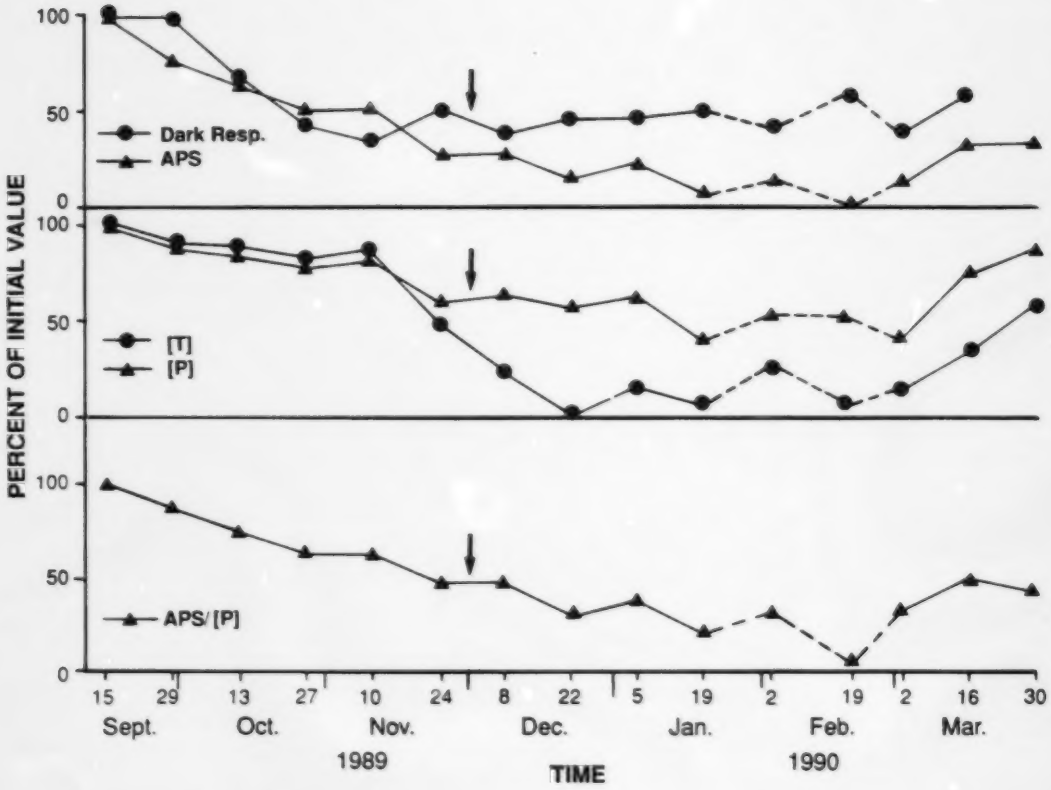


FIGURE 5.3. Example of a white spruce (seedlot 8534) baseline study, fall 1989 to spring 1990. Apparent photosynthesis (APS), dark respiration, variable fluorescence [P] and [T], and APS/[P] relative patterns determined at room temperature for white spruce seedlings brought in from the outside growing compound at Simon Fraser University. Data presented as percent of the initial mean value (n=5) on September 15, 1989. Note that on February 2 and 19 (dashed lines), seedlings were frozen into the PSB-313 styroblocks. They were removed for measurement after warm water was applied to them, so the data on these dates may be anomalous. Arrow indicates operational lifting date (November 29, 1989) for cold, dark storage of the commercial crop. (Data calculated from Brooke *et al.* 1990.)

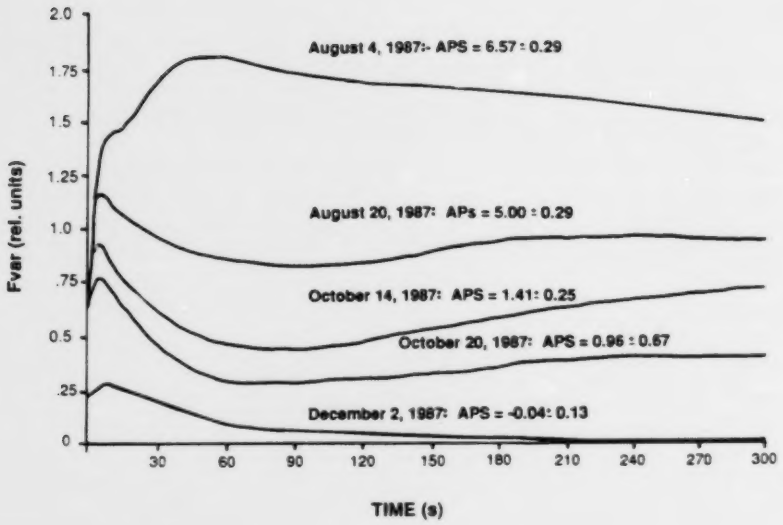

August 4, 1987:- APS = 6.57 : 0.29
August 20, 1987: APs = 5.00 : 0.29
October 14, 1987: APS = 1.41: 0.25
October 20, 1987: APS = 0.96 : 0.67
December 2, 1987: APS = -0.04: 0.13
Fvar (rel. units)
TIME (s)

FIGURE 5.4. An example of seasonal changes in F_{var} curves during inactivation of photosynthetic activity (APS) for white spruce seedlings (seedlot 8981) from early August to early December 1988. These patterns are associated with the winter hardening-off of the seedlings. (Data from Vidaver *et al.* 1988a.)

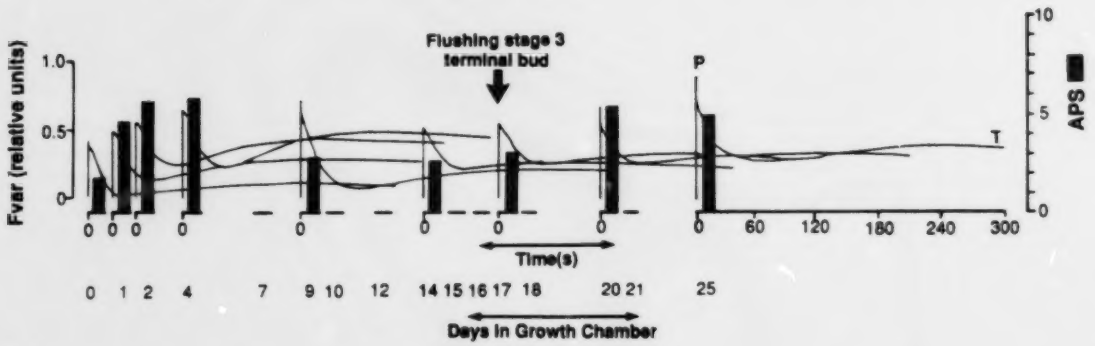


FIGURE 5.5. Example of F_{var} curves and photosynthesis (APS) rates for white spruce seedlings (seedlot 8981) following removal from cold, dark-storage. Following operational lifting on November 29, 1989, the 1+0 seedlings were cold, dark-stored (-2°C) for 47 days. On removal from storage, seedlings were held in the dark at about 2–4°C for 7 days before the 0 h room temperature F_{var} and APS assay, and then repotted. Following the 0 h assay, recovery of the seedlings to post bud-flush conditions occurred in a growth chamber (12 h photoperiod, 400 µmol m^{-2} s^{-1} PFD at canopy height, 20/15°C day/night temperature). To maintain moderate humidity around the shoot, a loose-fitted clear plastic bag was placed over each seedling in the growth chamber. All F_{var} time courses are normalized to F_o and like APS data averages of 10 seedlings. The APS rates are given as mg CO_2 g^{-1}odw·h^{-1}. (Data from Brooke *et al.* 1990.)

5.1.3 Relative contributions of new and the previous year's foliage to the fluorescence transient

White spruce (1+0)

Seedlot: 8981 (see Section 5.1.2)

Nursery, growing conditions: (see Section 5.1.2)

Test site: (see Section 5.1.2)

Duration of tests: February–March 1990

Results and interpretation: Newly flushed white spruce foliage has a pronounced [P] spike (Fig. 5.6). The photochemical activity of the combined foliage (previous year and new foliage) is intermediate between the F_{var} transients (see [P] and [T] levels) of the corresponding old- and newly flushed foliage-only assays. The APS rates of the intact and treated seedlings have a similar pattern (Fig. 5.6).

The high APS rates (oven-dry weight basis) of newly flushed white spruce needles suggest that their relative contribution to seedling carbon accumulation very quickly equals and soon surpasses that of the old needles.

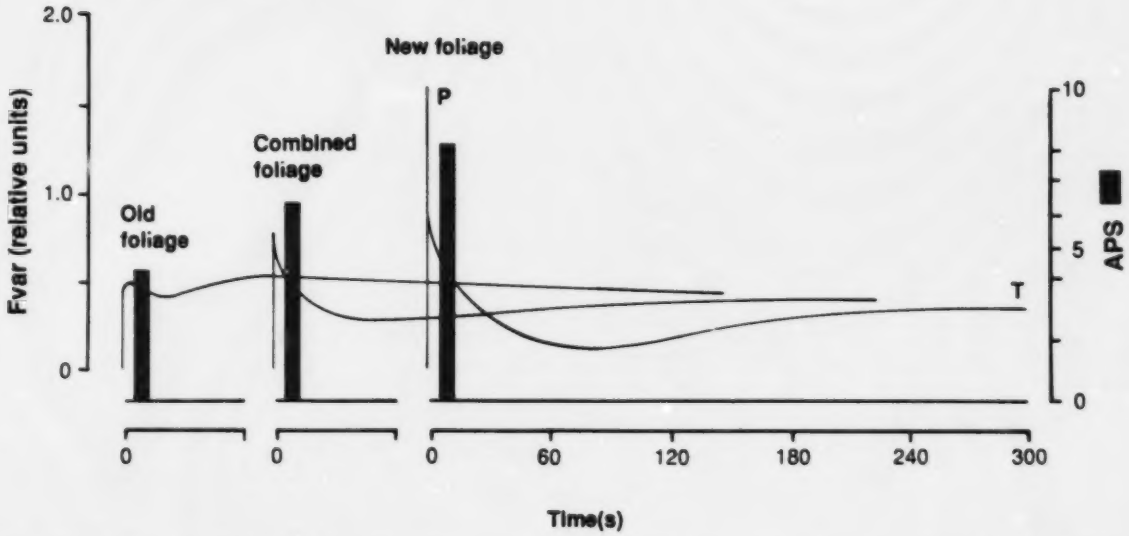


FIGURE 5.6. An example of mean F_{var} time courses and APS rates for white spruce seedlings (seedlot 8981) after 20 days in a growth chamber environment, following their removal from cold, dark storage (96 days). F_{var} curves and APS rates were obtained from the combined foliage of flushed seedlings (n=10) and then the photochemical activity was remeasured after the removal of either old foliage only (n=5) or new foliage only (n=5). For the treatment of the seedlings following their removal from cold, dark storage and APS units, see Fig. 5.5. (Data from Brooke *et al.* 1990.)

5.2 Long-Term Responses

Long-term cued events appear to link photochemical regulation to the season of the year.

5.2.1 Daylength-dependent photochemical regulation in white spruce

White spruce (2+0)

Seedlot: 8981, origin 58°N 120°W (750 m asl)

Nursery, growing conditions B C Ministry of Forests, Surrey, B.C. 49 °N 123°W, container-grown (PSB-313)

Test site: Seedlings were tested at Simon Fraser University, Burnaby, B.C. (49°N 123°W; 365 m asl) within 24 h of transport from the nursery

Duration of tests: August 1986- December 1988

Results and interpretation For the 1987 test (Fig. 5.7, 1987): Starting around mid-August, the F_{var} response began to decline By late October or early November, F_{var} reached a low level at which time apparent photosynthetic rates were approaching zero (Table 5.2).

For the 1986–1988 tests (Fig 5 7, 1986, 1988): Despite some differences, especially in mid-summer, the same trend of decline in apparent photosynthesis rate is seen in all 3 years (Table 5.2).

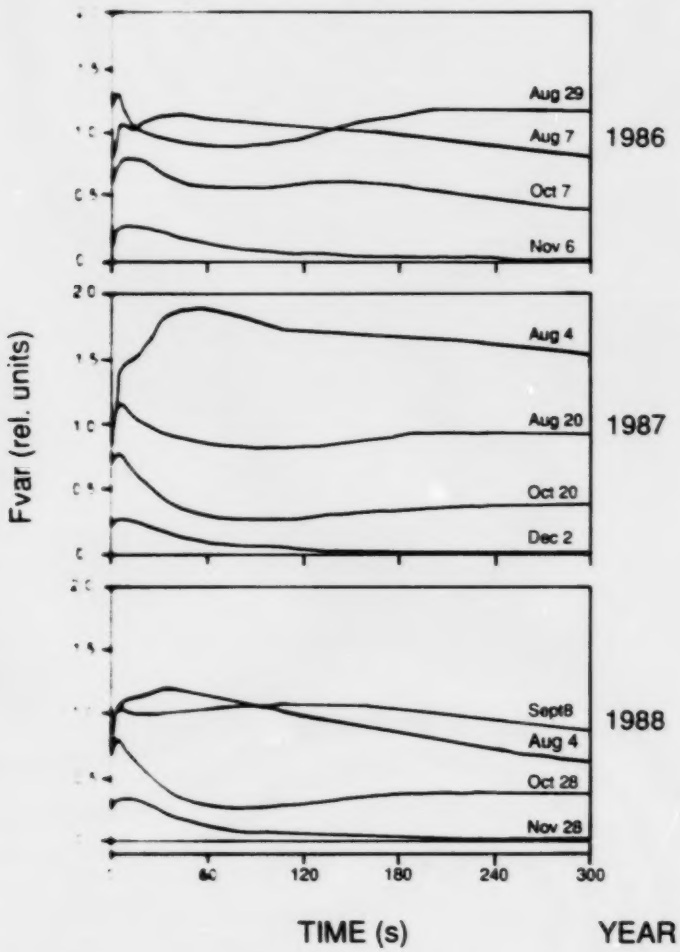


FIGURE 5.7. 1986, 1987, and 1988 F_{var} time courses for white spruce seedlings (seedlot 8981) during the August to December progression to physiological inactivation. (Data from Vidaver *et al.* 1989a.)

TABLE 5.2. Apparent photosynthesis rates for white spruce seedlot 8981 for 1986, 1987, and 1988, showing progression towards winter dormancy. Measurement dates correspond to the fluorescence time courses shown in Figure 5.7.

Year	Apparent photosynthesis (mg CO_2 h^{-1} g^{-1} oven dry weight needles)			
1986	August 7 9.66 ± 0.29[a]	August 29 8.27 ± 0.27	October 7 8.11 ± 0.48	November 6 1.54 ± 0 26
1987	August 4 6.57 ± 0.76	August 20 5.00 ± 0.29	October 20 0.96 ± 0.67	December 2 -0.04 ± 0.25
1988	August 4 3.25 ± 0.53	September 8 2.99 ± 0.58	October 28 trace[b]	November 28 trace[b]

[a] Mean and standard error n = 5.
[b] Rates were approximately 0.0.
(Data from: Vidaver *et al.* 1989a.)

White spruce exhibits daylength-dependent regulation of photochemical activity which may represent an adaptation that protects needles from photodamage during winter. This regulation would result in a gradual decline in F_{var} which coincides with progression toward winter dormancy. In timing, this form of regulation would appear to parallel bud set and the acquisition of cold-hardiness. Seedlings lifted on October 20, 1987, were judged to be cold hardy to -18°C (B.C. Ministry of Forests test). Operational lifting was on December 2. Much of the variation between the different years is attributable to weather differences before and at the time of measurement (see Fig. 5.8 for weather data and Section 5.3 on short-term responses). Long-term regulation has not been observed in coastal Douglas-fir or lodgepole pine.

5.2.2 Latitudinal provenance differences in F_{var} responses in white spruce

White spruce (2+0)

Seedlots: Northern, 8981, origin 58°N 120°W (750 m asl); southern 8534, origin 54°N 124°W (1060 m asl)

Nursery, growing conditions: 8981, 8534: B.C. Ministry of Forests, Surrey, B.C. (49°N 123°W), container-grown (PSB-313); 4073, B.C. Ministry of Forests, Red Rock, B.C. (54°N 123°W)

Test site: Seedlings were tested at Simon Fraser University, Burnaby, B.C. (49°N 123°W; 365 m asl) within 24 h of transport from the nursery

Duration of tests: August 1986–December 1988

Results and interpretation: F_{var} decline appeared earlier in the more northern provenance seedlings than in the southern provenance even though seedlings grew side-by-side in the same nursery (Fig. 5.9). Although less than 4° of latitude (Fig. 5.1) separate the northern from the southern provenance, the progression appears to lag about 2–3 weeks in 8534 (see Fig. 5.10). This relationship persisted over the 3 years of testing (Fig. 5.9).

Although both seedlots apparently displayed daylength-dependent regulation, it began earlier and progressed more rapidly in the northern provenance seedlings. This difference appears to result from the adaptations which protect seedlings from photodamage. The earlier decline in 8981 seedlings is probably a genotypic response to the more rapid onset of winter at higher latitudes. This timing by different spruce provenances may help to account for geographical limitations in the success of some outplants. The data suggest that the dates on which seedlings are lifted for storage should be further investigated.

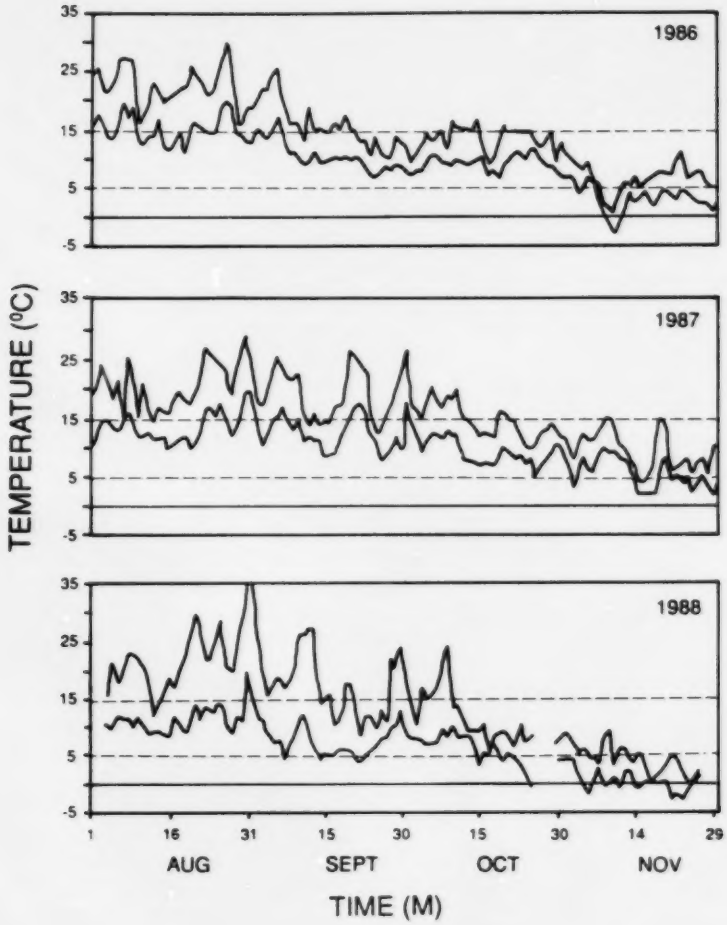


FIGURE 5.8. Maximum and minimum temperature patterns for 1986, 1987, and 1988 at the outside growing compound at Simon Fraser University, which correspond to the 1986, 1987, and 1988 F_{var} time courses in Fig. 5.7. Note the uniformity in September to October 1986 data compared to 1987 and 1988. The +5°C line gives some indication of the chilling units received by seedling stock in the outside compound at Simon Fraser University. (Data from Vidaver *et al.* 1989a.)

5.2.3 Recovery of photochemical activity in seedlings following cold, dark storage

White spruce (2+0)

Seedlot: 4073, origin 53°46'N 121°30'W (1190 m asl)

Nursery, growing conditions: B.C. Ministry of Forests, Surrey, B.C. (49°N 123°W), container-grown (PSB-313), lifted and stored at -2°C in December 1987

Test site: Seedlings were tested at Simon Fraser University, Burnaby, B.C. (49°N 123°W; 365 m asl) within 24 h of transport from the nursery

Duration of tests: May 1988

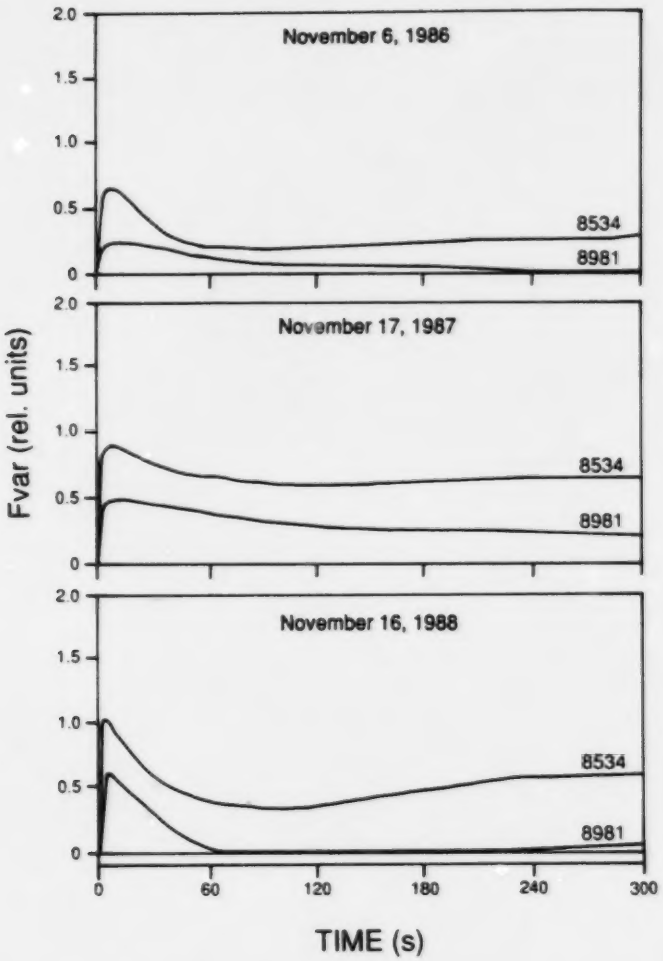


FIGURE 5.9. Comparison of 1986, 1987, and 1988 F_{var} time courses during photosynthetic inactivation for a northern (seedlot 8981) and more southern (seedlot 8534) provenance of white spruce. Note that in early to mid-November each year, the more northern provenance had progressed further to complete inactivation. For seedlot locations, see Fig. 5.1. (Data from Vidaver *et al.* 1989a.)

Results and interpretation: Seedlings showed indications of F_{var} recovery (Fig. 5.11) within 24 h and by 48 h had increased to the level expected from white spruce seedlings in mid-growing season (Fig. 5.7). These seedlings subsequently scored more than 4 on the standard Burdett (1979) root growth index (RGI) scale.

Properly lifted and stored seedlings should remain physiologically inactive and display low levels of CO_2 exchange and F_{var} during storage. When removed from storage, vigorous seedlings having met their chilling requirement should rapidly recover their photosynthetic activity. This would be seen as a return to the typical F_{var} response curve. Delayed or incomplete recovery of F_{var} can be considered an indication of reduced seedling vigour. Seedlings having poor F_{var} recovery also had low RGI scores (Table 5.3).

TABLE 5.3. Maximum F_{var} values for white spruce seedlings 48 h after removal from cold, dark storage, and their corresponding RGI scores

Seedlot	F_{var} (n = 6)	RGI (n = 16)
8503	0.37 ± 0.04[a]	0.63 ± 0.50[a]
8872	0.53 ± 0.01	1.06 ± 0.44
8533	0.63 ± 0.20	1.13 ± 0.02
4073	1.28 ± 0.16[b]	4.88 ± 0.15

[a] Mean ± standard error.
b m = 5
(B.C. Ministry of Forests, Silviculture Branch, carried out the RGI tests. Data from: Vidaver *et al.* 1989a.)

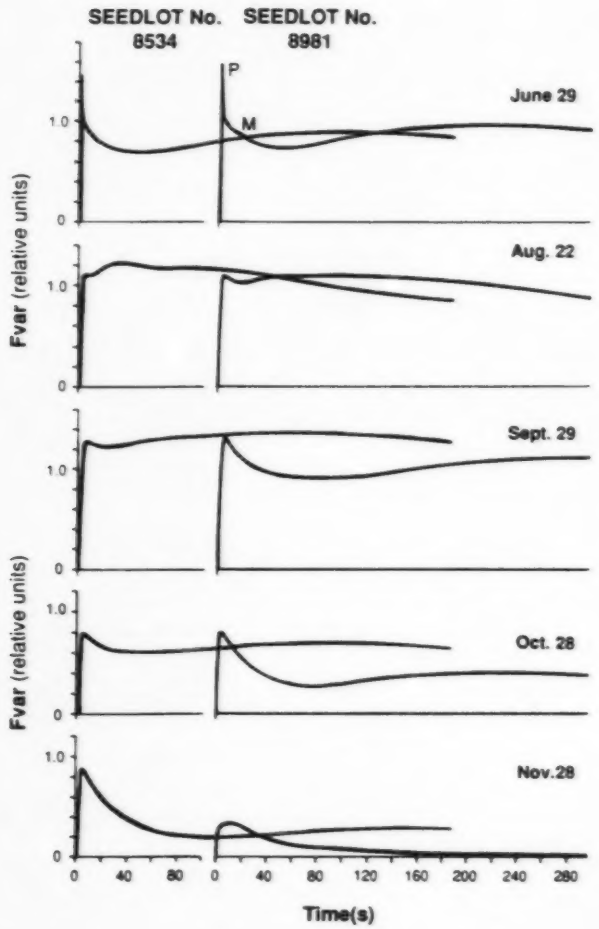


FIGURE 5.10. Latitudinal provenance differences in photochemical regulation in white spruce seedlings (seedlots 8981 and 8534). Note that the more northern seedlot (8981) begins inactivation earlier (September 29), and that the more southern seedlot (8534) only reaches about the same level of inactivation by November 28 as reached by the more northern seedlot (8[illegible]) 1 month earlier (October 28). For seedlot locations, see Fig. 5.1. (Data from Brooke *et al.* 1989.)

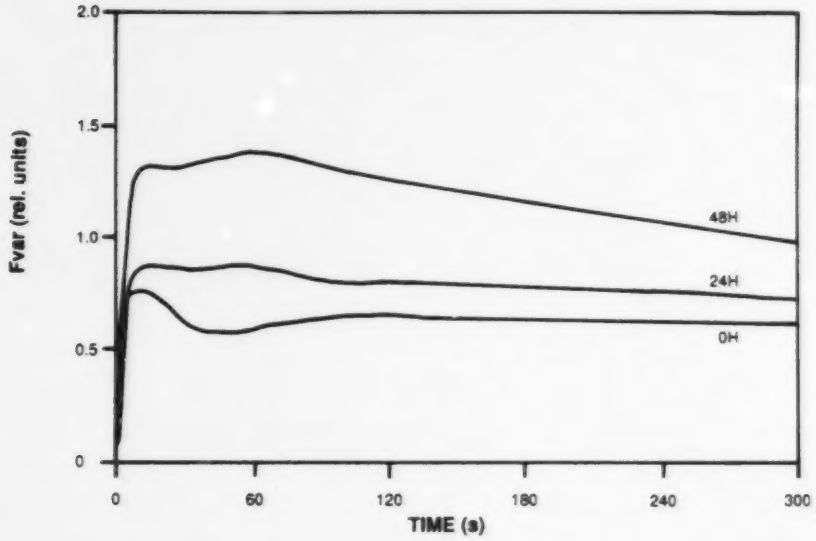


FIGURE 5.11. F_{var} recovery of white spruce seedlings (seedlot 4073) during the first 48 h after removal from cold, dark storage. Seedlings tested were repotted, watered, and equilibrated to room temperatures following their removal from storage. Note that at 48 h (top curve), the F_{var} time course shows almost full recovery to levels expected from fully active seedlings. RGI determinations usually take 1 or more weeks (see corresponding data for seedlot 4073 in Table 5.3). (Data from: Vidaver *et al.* 1988a.)

Douglas-fir

Seedlot: Origin unknown, part of an operational outplant "blind test" of apparently poor stock

Nursery growing conditions: Unknown, container grown (PSB-313), lifted and stored at -2°C in the winter of 1987

Duration of test: Two weeks, late spring 1988

Results and interpretation: These seedlings showed clear indications of recovery over the full 8 days of the test (Fig. 5.12).

While the reactivation of the photosynthetic system is satisfactory, as indicated by the rapid progressive changes in the pattern of F_{var} towards that of typical non-stress, mid-growth season curve form, the corresponding RGI score was below 1.0 . However, the subsequent outplant survival was deemed to be "moderate to satisfactory" (i.e., >80% survival after 1 year). Thus, the F_{var} assay may provide a means of reducing the rejection level set by a single assessment decision such as RGI (see Hawkins and Binder 1991).

5.3 Short-Term Responses

The F_{var} response in all temperate conifers tested to date is reversibly affected by changes in temperature, water availability, or other environmental factors which may lead to stress.

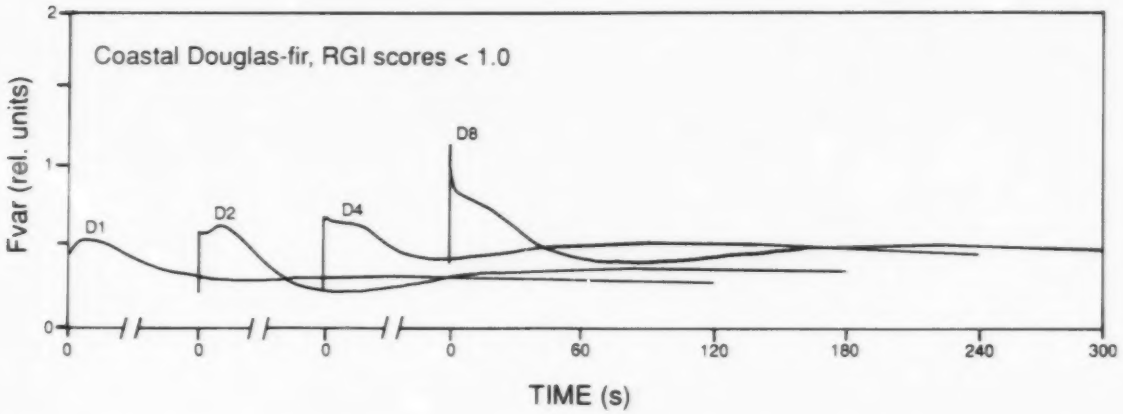


FIGURE 5.12. F_{var} time courses for coastal Douglas-fir seedlings for 1, 2, 4 and 8 days following their removal from cold, dark storage.

5.3.1 Short-term effects of water deficits on photochemical activity in seedlings during the growing season

White spruce (2+0)

Seedlot: 8981, origin 58°N 120°W (750 m asl)

Nursery, growing conditions: B.C. Ministry of Forests, Surrey, B.C. (49°N 123°W), container-grown (PSB-313)

Test site: Seedlings were tested at Simon Fraser University, Burnaby, B.C. (49°N 123°W; 365 m asl)

Duration of tests: June, July and August 1987

White spruce has very sensitive stomatal responses to environmental moisture conditions. Vapour pressure deficits (VPD) appear to be a major factor controlling their activity (Goldstein *et al.* 1985). For provenances of white spruce near tree-line in northern Alaska, their data indicate that at VPD's above 1.0 kPa, stomata are essentially closed and that stomatal conductance declines markedly as VPD's increase above 0.2 kPa (Goldstein *et al.* 1985). Temperature and VPD data for our controlled watering and water deficit experiments are given in Table 5.4.

5.3.1.1 Water deficits in unwatered seedlings

White spruce seedlings (Seedlot No. 8981), growing outside and dependent on rain for water, showed a decline in F_{var} in response to VPD's. On June 7, 1987, VPD's ranged from 1.0 to 2.0 kPa for most of the day. With a pre-sunrise VPD of 0.17 kPa on June 8 and a VPD near only 0.5 kPa (Table 5.4) at the time of assay, both F_{var} and APS measurements indicated minimal water stress (Fig. 5.13). On June 25, with last precipitation several days earlier and following a day with VPD's between 1.2 and 1.8 kPa, temperatures were over 23°C and the VPD was 1.1 kPa at the mid-morning assay. The F_{var} had declined noticeably at this assay and the APS rate was less than half that on June 7 (Fig. 5.13). A pre-sunrise VPD of 0.2 on June 25, the high temperature and VPD at the time of measurement suggest that the white spruce seedlings did not recover from the high VPD's the day before. On July 6, after several days with relatively low VPD's throughout the day, both F_{var} and APS had recovered (Fig. 5.13).

TABLE 5.4. Vapour pressure deficits (VPD in kPa) and corresponding temperatures (°C) at the Simon Fraser University test site for pre-sunrise, mid-morning, and afternoon maximum conditions. The mid-morning time corresponds to the time of fluorescence measurements made in the 1987 water deficit experiments.

	Pre-sunrise		Mid-morning		Afternoon maximum		
Date[a]	Temp.	VPD	Temp.	VPD	Temp.	VPD	See Figure –
June 2	6.0	0.06	14.5	0.53	20.0	1.22	
3	11.0	0.32	19.5	1.06	27.0	2.25	
4	13.5	0.31	24.5	1.29	27.3	2.03	
5	12.0	0.14	13.0	0.18	18.2	0.56	
6	9.0	0.00	15.0	0.38	20.0	1.05	
7	11.0	0.07	21.8	1.12	27.0	2.00	
8*	12.0	0.16	17.0	0.46	14.5	0.16	5.13
June 19	11.6	0.34	20.0	0.80	25.7	1.55	
20	15.0	0.26	17.0	0.62	16.0	0.09	
21	10.3	0.00	10.7	0.00	14.3	0.46	
22	9.0	0.01	12.0	0.10	17.0	0.81	
23	9.0	0.12	12.5	0.26	20.8	1.30	
24	10.0	0.22	23.0	1.26	26.0	1.85	
25*	8.8	0.19	23.2	1.08	30.0	2.67	5.13
June 30	18.5	0.47	25.5	1.18	32.8	2.59	
July 1	17.5	0.40	25.5	1.14	27.0	1.50	
2	13.3	0.05	15.0	0.15	18.2	0.54	
3	13.8	0.10	14.0	0.00	20.8	0.71	
4	13.0	0.00	14.5	0.15	15.0	0.27	
5	11.0	0.00	11.0	0.03	12.2	0.06	
6*	9.2	0.09	12.0	0.21	17.3	0.79	5.13
July 8	9.8	0.24	10.0	0.00	14.2	0.45	
9	9.2	0.06	11.2	0.13	18.5	0.81	
10	9.7	0.06	18.7	0.71	25.8	1.83	
11	15.0	0.34	22.0	0.87	28.2	2.03	
12	15.0	0.34	22.5	0.95	29.8	2.22	
13	15.5	0.28	22.8	1.06	29.4	2.05	
14	15.0	0.17	22.0	0.79	28.0	2.12	
15	10.3	0.05	15.0	0.43	22.3	1.40	
16*	9.8	0.00	17.8	0.57	22.1	1.20	5.14
17*	12.9	0.24	23.3	1.43	29.6	2.40	5.14
18*	15.2	0.17	18.5	0.64	28.5	2.02	5.15/5.16
19*	13.9	0.08	14.8	0.08	20.1	0.59	5.15/5.16
20*	13.5	0.08	17.5	0.40	24.5	1.32	5.16/5.18
21*	14.0	0.16	22.0	0.85	29.0	2.24	5.16/5.18
22*	14.5	0.13	15.8	0.18	20.2	0.62	5.16/5.18
23*	13.5	0.08	18.0	0.37	24.6	1.24	5.18
24	14.6	0.00	21.5	0.36	25.2	1.06	
25	13.8	0.00	14.8	0.12	15.8	0.11	
26*	12.8	0.03	18.5	0.30	21.7	0.73	5.17/5.18
27*	13.2	0.00	16.8	0.23	22.3	1.18	5.17/5.18
August 3	13.8	0.19	23.0	0.98	28.5	2.06	
4	14.7	0.07	18.2	0.34	26.0	1.61	
5	10.1	0.01	15.0	0.12	23.8	1.15	

(Continued)

TABLE 5.4. (Continued)

Date[a]	Pre-sunrise		Mid-morning		Afternoon maximum		
	Temp.	VPD	Temp.	VPD	Temp.	VPD	See Figure –
6	13.8	0.10	18.0	0.31	26.5	1.56	
7	14.2	0.06	23.0	0.84	28.0	1.93	
8	15.0	0.15	22.5	0.68	29.0	2.04	
9	15.2	0.19	23.5	0.81	29.0	1.88	
10*	12.7	0.07	14.0	0.11	18.3	0.42	5.19
11*	11.9	0.00	16.0	0.18	25.5	1.53	5.19

[a] (*) indicates date of a fluorescence measurement. On the date of measurement, seedlings were taken from the test site compound at Simon Fraser University at mid-morning, assayed for F_{var} and APS, and returned immediately to the compound and watered. Seedlings which were on a fixed experimental watering cycle of 48, 96, or 144 h were located under a plastic canopy that prevented any precipitation from reaching the seedlings. On the watering days, seedlings were immersed in water up to approximately 1 inch from the top of the styroblock. Block weights (not shown) were taken before and after watering.

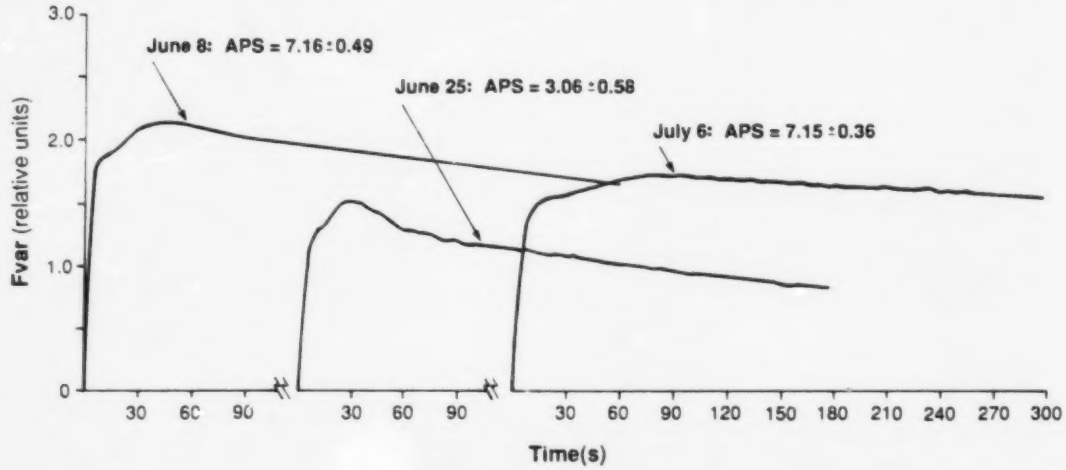


FIGURE 5.13. Changes in the F_{var} time courses and apparent photosynthetic (APS) rates of white spruce seedlings (seedlot 8534) to natural water stress and recovery during the 2nd year of their production cycle. From June 1 to July 15, there were large temperature and relative humidity changes at the growing site, resulting in increased water stress on June 25. At the time of F_{var} assay on June 25, VPD was approximately 2 times higher than the corresponding VPD on June 8 (see data in Table 5.4). (Data from: Vidaver *et al.* 1988a.)

5.3.1.2 Water deficits in regularly watered seedlings

- 48 h watering cycle:

Seedlings last watered on July 14, 1987, exhibited low to moderate water deficits within 2 days (Fig. 5.14, July 16). Following watering immediately after the July 16 assay, only slight recovery occurred to the time of assay the next day (July 17) (the VPD pattern including the pre-sunrise VPD of 0.24 kPa remained high). A continued high VPD pattern (July 17 to mid-morning assay July 18) (Table 5.4) resulted in a F_{var} decline on July 18 before seedling re-watering (Fig. 5.15). With re-watering and VPD's falling from 2.0 kPa (July 18) to 0.08 at pre-sunrise and to the time of assay on July 19, F_{var}, APS, and WP_{xylem} show recovery (Fig. 5.15).

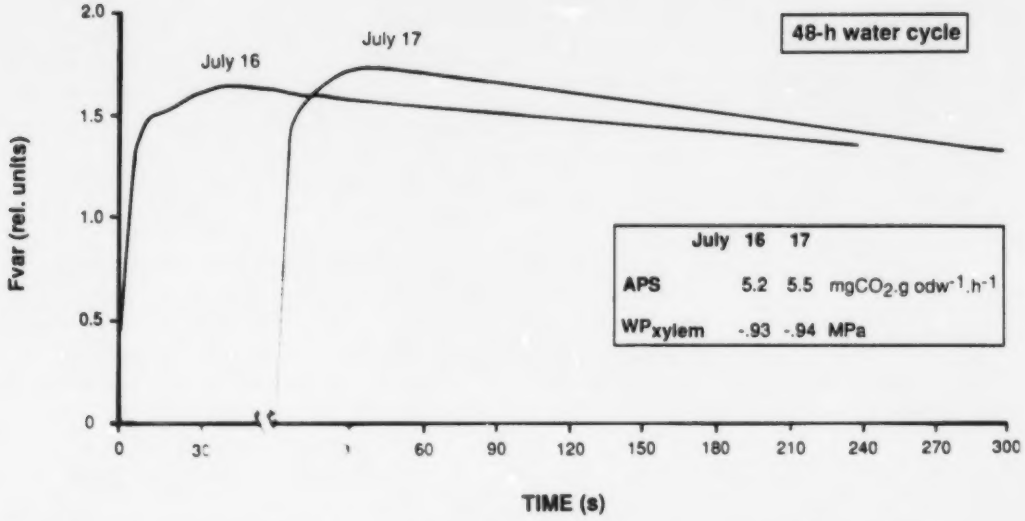


FIGURE 5.14. F_{var} time courses for white spruce seedlings (seedlot 8981) for 48 h without water (July 16) and their recovery 18 h after re-watering (July 17). Although VPD's were relatively high during the daytime for the 48 h when the plants were without water, there was no VPD at pre-sunrise preceding the F_{var} measurement on July 16 at mid-morning. The seedlings are less water-stressed than those on June 25 (Fig. 5.13) and on July 18 (Fig. 5.15). The July 17 F_{var} curve shows some recovery following re-watering, but note that the VPD's remained high with little overnight recovery following the July 16 F_{var} measurement (see data Table 5.4). (Data from: Vidaver *et al* 1988b)

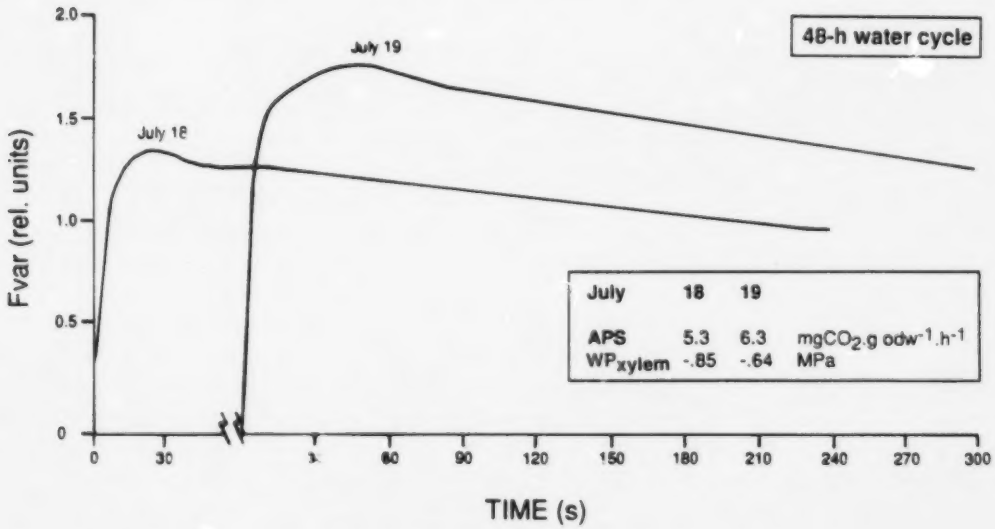


FIGURE 5.15. F_{var} time courses for white spruce seedlings (seedlot 8981) for 48 h without watering (July 18) and their recovery on July 19, 18 h after re-watering. A high VPD occurred on July 17, and with some deficit still remaining at pre-sunrise, seedlings measured July 18 show more water stress than seedlings measured on July 16 (see Fig. 5.14). There is a more pronounced "recovery" following re watering immediately after the F_{var} assay on July 18 (see data on Table 5.4) because of the lower VPD's at pre-dawn and up to the time of measurement on

- 96 h watering cycle:

Seedlings last watered on July 14 exhibited a moderate decline in F_{var} on July 18 after 96 h without water (Fig. 5.16). This decline was not overcome on July 19, despite re-watering after the assay on the previous day. Recovery was evident 42 h after watering on July 20 which coincided with a period of moderate to low VPD's on the 19th (see Table 5.4). With increasing VPD after the assay on the 20th, seedlings last watered 3 days earlier again showed signs of water deficit on July 21 (0.85 kPa VPD at assay). Recovery of the seedlings' F_{var} response is evident on July 22 (Fig. 5.16), which coincides with low VPD's of 0.13–0.18 kPa to mid-morning. Re-watering on the 22nd after the F_{var} assay enabled the recovery of F_{var} to persist for 96 h without re-watering until July 26 (Fig. 5.17). Mid-morning VPD's for this 96-h period did not exceed 0.4 kPa and maximum afternoon VPD's of between 1.0 and 1.3 KPa were reached on only two occasions (see Table 5.4). Re-watering on July 26 after the F_{var} assay resulted in little further change in F_{var} response and only a slight increase in APS rate (Fig. 5.17).

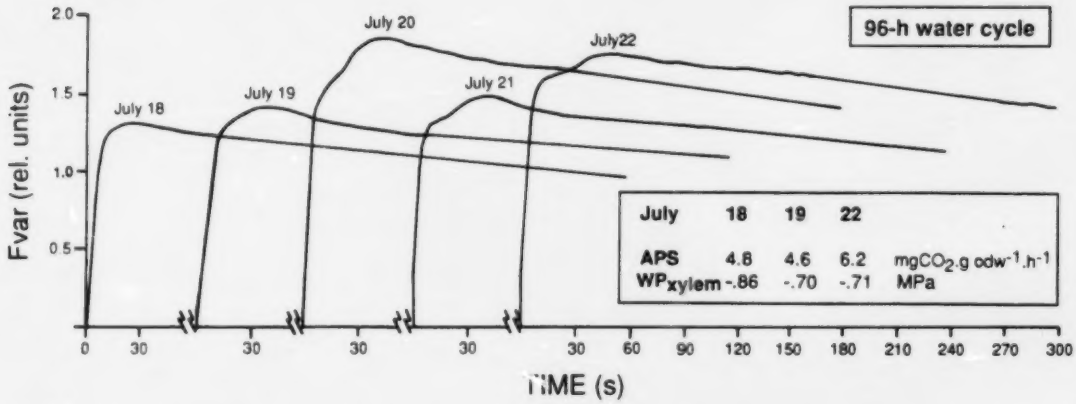


FIGURE 5.16. F_{var} time courses for white spruce seedlings (seedlot 8981) for 96 h without watering (July 18) and their 96-h recovery responses after re-watering on July 18 immediately following the F_{var} assay. Recovery took about 48 h (July 20 curve), but some water stress was again evident on July 21 following the relatively high VPD on the previous day and lower pre-sunrise recovery of VPD (July 21) preceding the F_{var} assay. Although a high VPD occurred in the afternoon of July 21, VPD's were much reduced the following day. At the mid-morning F_{var} assay time (July 22), the VPD was only about one-fifth the VPD deficit at the assay time on July 21 (see data on Table 5.4). (Data from: Vidaver *et al.* 1988b.)

- 144 h watering cycle:

Seedlings last watered on July 14 showed relatively severe indications of water deficit on July 20 (see F_{var} curve, APS, and WP_{xylem} data in Fig. 5.18) after 144 h without water. During this period, seedlings initially experienced a rise in VPD followed by a moderating period (see Table 5.4). Watering on July 20 after the F_{var} assay resulted in some recovery on July 21, followed by further recovery on July 22–23. Except for July 21, the period July 20–27 was a period of moderate VPD's. Nevertheless, the lowered F_{var} response on July 26 (Fig. 5.18) shows how daytime temperatures between 15 and 29°C and lack of water for 6 days affects seedlings growing in a small peaty soil volume. Re-watering on July 26 following the F_{var} assay resulted in some improvement in the APS rate, but little in the F_{var} response on July 27 (Fig. 5.18).

5.3.1.3 Long-term water withholding

The F_{var} curve for August 10 (Fig. 5.19) shows a severe water stress response for seedlings last watered 11 days earlier. This period without water had an average mid-morning VPD of 0.46 and afternoon average maximum VPD of 1.49, with temperatures ranging from 14 to 29°C (Table 5.4).

After placing the styroblock containers of seedlings in a water bath for several hours after the F_{var} assay on August 10, the F_{var} response pattern on August 11, about 18 h after re-watering, shows that the seedlings still have a slight recovery response to re-watering, especially when it coincides with low VPD conditions conducive to recovery.

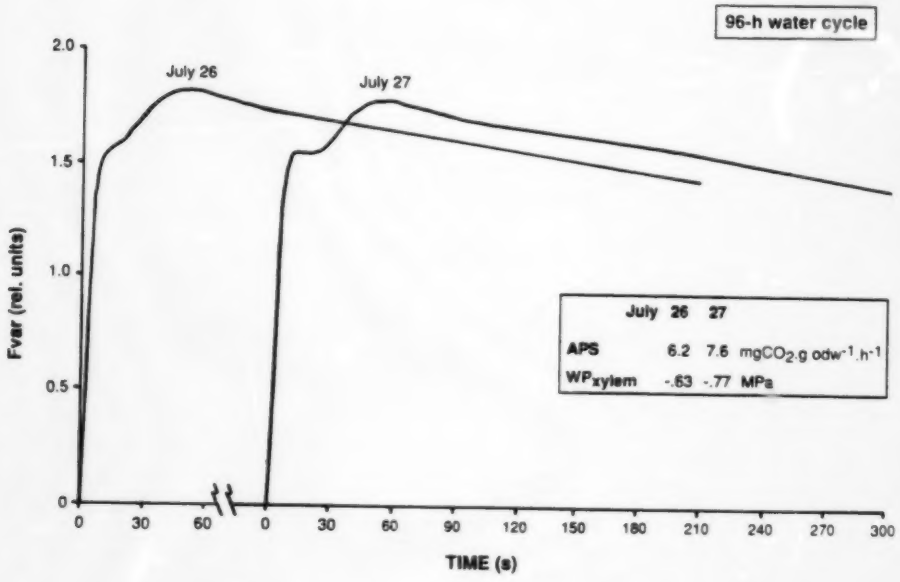


FIGURE 5.17. F_{var} time courses for white spruce seedlings (seedlot 8981) on a 96-h watering cycle. As the VPD's remained low for most of the 96 h without watering up to the July 26 assay, re-watering resulted in little change in the F_{var} response on July 27 (see Table 5.4 for VPD data). (Data from: Vidaver *et al.* 1988b.)

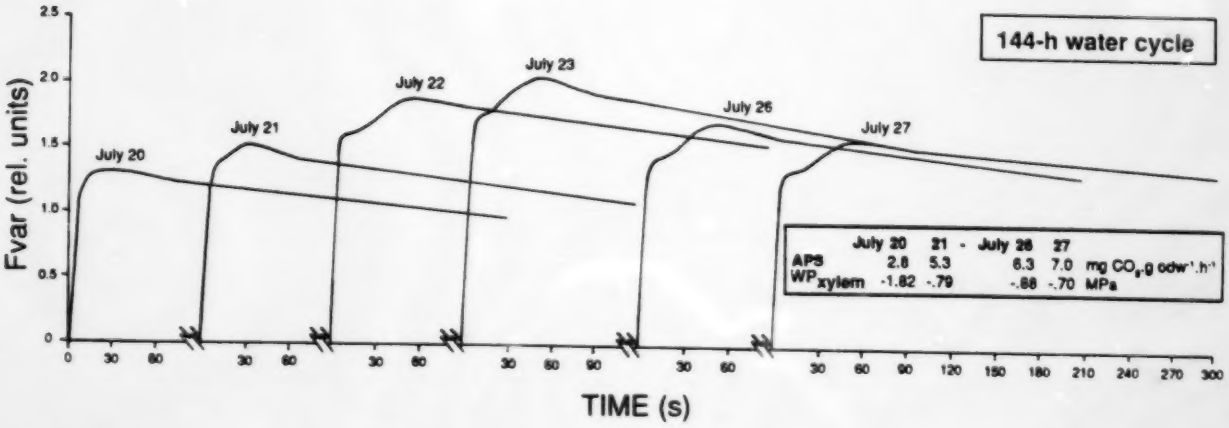


FIGURE 5.18. F_{var} time courses for white spruce seedlings (seedlot 8981) on a 144-h (6-day) watering cycle. Maximum recovery from water stress on this longer cycle without water took nearly 3 days (July 23 curve) in contrast to the 2 days for seedlings on a shorter re-watering cycle (see Fig. 5.17; Table 5.4 has the corresponding VPD data). (Data from: Vidaver *et al.* 1988b.)

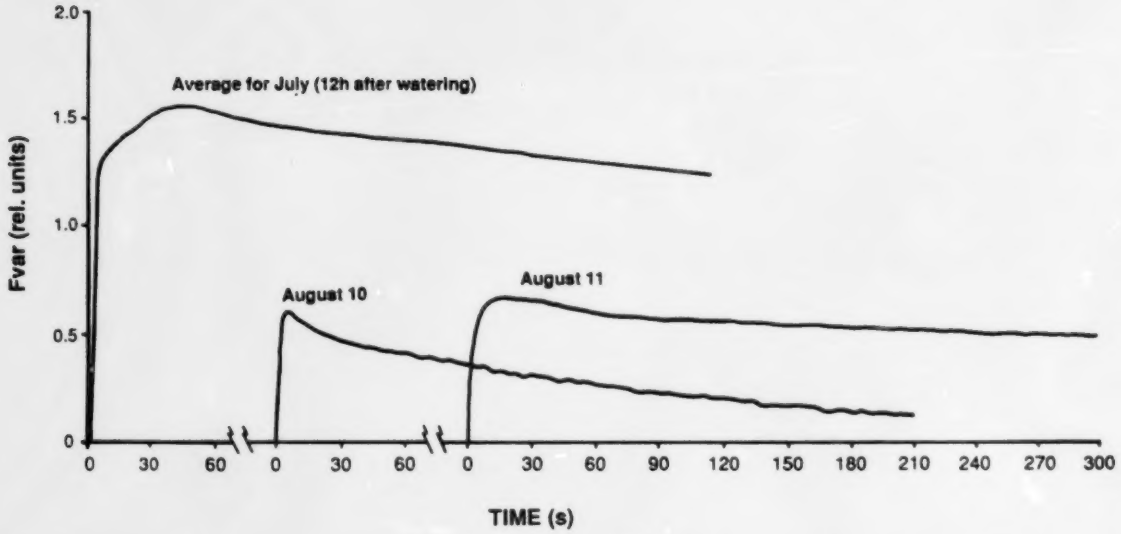


FIGURE 5.19. F_{var} time courses for white spruce seedlings (seedlot 8981) showing the response following 11 days without water (August 10) and the recovery on August 11, 12 h following re-watering. Note the extreme change between the F_{var} transient for August 10 and the average after re-watering transient for July. Following re-watering on August 10, some recovery from water stress occurred in response to lower VPD's (see Table 5.4). (Data from: Vidaver *et al.* 1988b.)

5.3.1.4 Interpretation of F_{var} response to water deficits

In response to water deficits, F_{var} in conifer seedlings may undergo a decline which persists until the deficit is alleviated. This decline may represent short-term regulation of photochemical activity, which could be a mechanism to prevent photodamage when photosynthetic biochemistry (CO_2 assimilation) is inhibited by water stress. The data shown here indicate that even with regular watering, on occasions with high daytime VPD and little opportunity for seedlings to recover overnight, a decrease in apparent photosynthesis rate can occur (see Goldstein *et al.* [1985] and Binder *et al.* [1988] for additional data). Persistent loss of photosynthetic potential can result in a decrease in cumulative growth during the seedling production cycle. The fact that the photosynthesis rates of seedlings declined with high VPD's, even in saturated container blocks, indicates that when seedlings have confined roots in a small soil cavity volume, root water uptake cannot keep up with shoot transpirational losses. Misting or fogging during high VPD periods may help to alleviate such problems.

5.3.2 Elevational temperature-dependent response differences

Douglas-fir (2+0)

Seedlots: Coastal: high elevation (760+ m asl) 6399 origin, 49°N 122°W; low elevation (152 m asl) 1273 origin, 49°N 122°W

Nursery, growing conditions: B.C. Ministry of Forests, Surrey, B.C. container-grown (PSB-313)

Test site: Seedlings were maintained in compound at Simon Fraser University, Burnaby, B.C. (49°N 123°W; 365 m asl) for the duration of the tests

Duration of test: August 1988–March 1989

Results and interpretation (see Fig. 5.2): Neither high nor low elevation seedlings showed any indication of daylength-dependent regulation in the F_{var} assays. Both seedlots indicated an apparent temperature-dependent regulation (bar graphs in Fig. 5.2 to the right of the curves display temperature data for 4 days before and on the date of measurement). The F_{var} pattern of the low elevation seedlings appeared more sensitive than the high elevation seedlings to the declining temperatures in the fall. However, there was little difference between the seedlots from mid-December through March, although fluctuations occurred with temperature changes in both seedlots over this period.

Although there was no indication of long-term daylength-dependent regulation in these seedlings, F_{var} showed that they do respond to temperature variations. These responses apparently reflect a decline in photochemical activity as temperatures approach freezing levels.

Such a response could be adaptive in that photochemical activity may be much reduced at temperature extremes to protect the plant from photodamage when CO_2 assimilation is inhibited. Provenance differences in Douglas-fir are an important factor to be considered in nursery seedling production and reforestation of logged sites.

PART II

ENVIRONMENTAL AND PHYSIOLOGICAL BASES OF CHLOROPHYLL FLUORESCENCE ASSESSMENT

6 PHOTOSYNTHESIS AND THE ORIGIN OF VARIABLE CHLOROPHYLL FLUORESCENCE

To understand the origin of variable chlorophyll fluorescence, as well as the significance of the components of the F_{var} time course and how it can be affected by environmental factors, it is necessary to examine the overall photosynthetic process and the steps that it comprises.

6.1 Three Phases of Photosynthesis

In green plants, photosynthesis can be separated into three distinct phases: light harvesting, photochemistry, and biochemistry (Fig. 6.1). Each of these phases comprises several steps which differ in their rates and sensitivity to environmental factors. For photosynthesis to proceed effectively and without resulting in damage to chloroplast structures, each phase and its component steps must remain in balance with all the others.

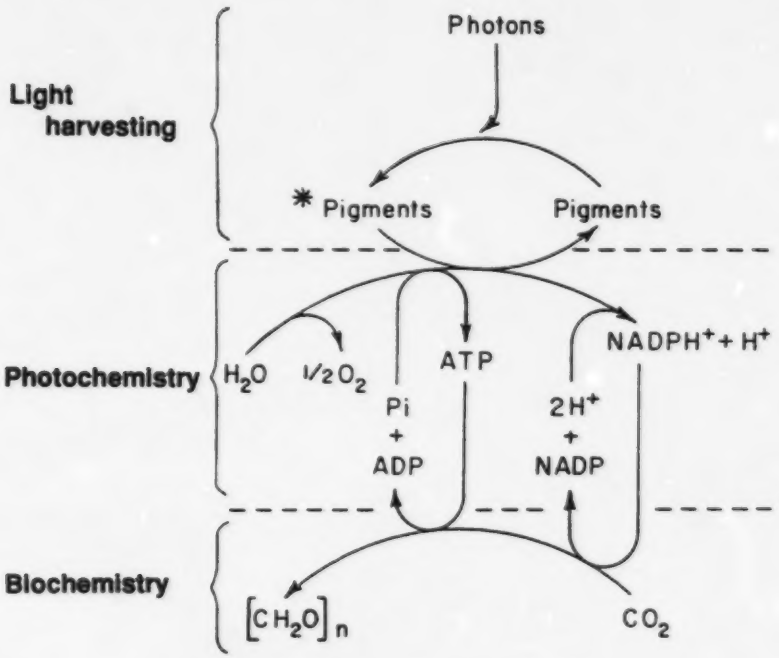


FIGURE 6.1. Scheme representing the light harvesting or absorption, photochemical, and biochemical phases of photosynthesis. Light absorption causes the excitation of thylakoid pigments (*), leading to a transfer of electrons from special trap chlorophylls to the primary acceptor molecules. The transfer of electrons between intermediate carriers of the electron transport chain leads to the production of $NADPH^+$ and a proton gradient driven synthesis of ATP. Photosynthetic biochemistry can proceed in the dark and requires three ATP's and two $NADPH^+$'s for each CO_2 assimilated. (Adapted from Nobel 1983.)

As used in the F_{var} analysis system, the fluorescence time course (see Section 2.2; Fig. 2.4) is measured after a period of dark pretreatment. This induces an unbalanced state in the various steps of photosynthesis. *When the dark pretreated sample is suddenly illuminated, the transient changes in the F_{var} time course reflect the emerging balance of the different steps.* The F_{var} time course can be best interpreted in terms of interactions between the photosynthetic sub-processes. Some of these contribute to an increase and others to a decrease in fluorescence emission.

The major steps of photosynthesis, their effect on F_{var}, and their role in overall photosynthesis are outlined below and displayed schematically in Figure 6.2.

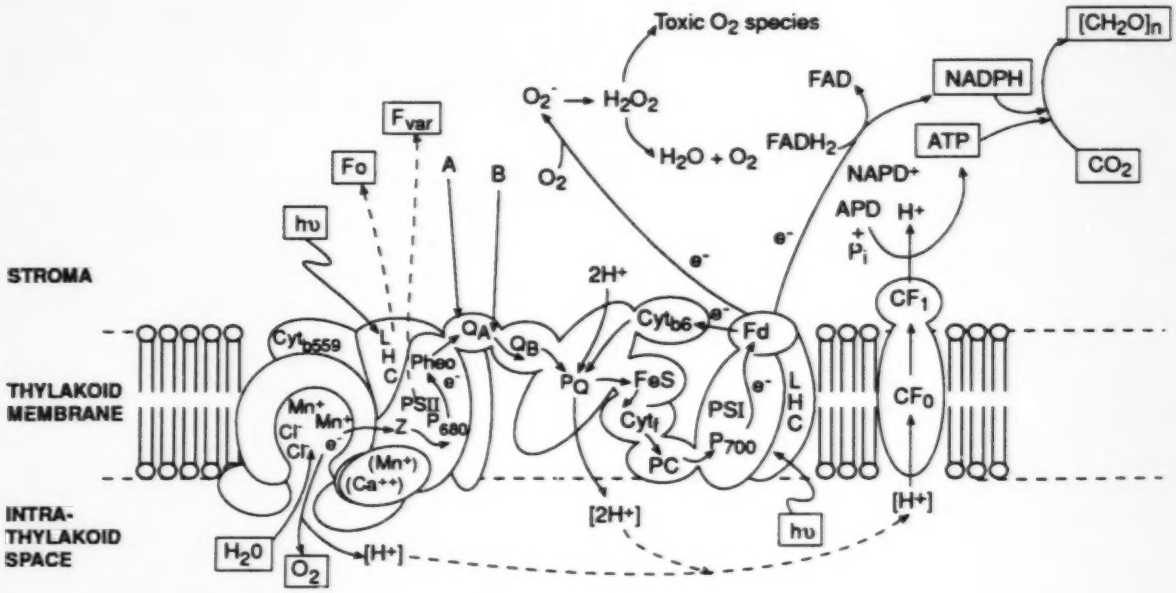


FIGURE 6.2. Schematic summary of the structural components and the physical and biochemical processes of the chloroplast thylakoid membrane, leading to assimilation of CO_2. Water-splitting, electron transport, and cyclic and non-cyclic electron flow are represented. Symbols and abbreviations are: LHC, light harvesting pigment complex; Z, the primary donor of the oxidizing side of PSII; P_{680}, the PSII reaction centre; Pheo, acceptor of exciton from P_{680}; Q_A, the PSII primary acceptor; Q_B, the secondary acceptor; PQ, plastoquinone; Cyt_f, cytochrome f; Fe-S, iron-sulphur (Rieske protein); PC, plastocyanin; P_{700}, the PSI reaction centre; Fd, ferredoxin; Cyt_{b6}, cytochrome b_6; Cyt_{b559}, cytochrome b_{559}; $NADP^+$, nicotinamide-adenine dinucleotide phosphate (+$2H^+$, reduced); FAD, flavine adenine dinucleotide (+$2H^+$, reduced); CF_o, CF_1, components of proton gradient driven ATP synthesis system. Cyclic electron flow occurs when electrons from the PSI acceptor are recycled to PQ via Cyt_{b6}.
[A] Block e^- flow before Q_A results in a decrease in F_{var}.
[B] Block e^- flow here, by DCMU, for example, or at any point further on, or impede CO_2 reduction in any manner, will result in an increase in F_{var}.
Details depicted here are further explained and discussed throughout Section 6 of the text.

The overall photosynthetic process can be expressed by the equation:

$$6CO_2 + 6H_2O + 12NADPH^+ + 18ATP \longrightarrow C_6H_{12}O_6 + 6O_2 + 12NADP^+ + 18ADP + 18Pi$$

Light energy harvested by thylakoid pigment molecules is used to produce $NADPH^+$ and ATP which are required at a ratio of 2:3 for the assimilation of a CO_2 molecule. Six CO_2's are required to produce a sugar molecule.

6.1.1 Light harvesting

Light-harvesting chlorophyll molecules are arranged in an organized protein matrix on the outer surface of the thylakoid (sac-like structures inside chloroplasts) membrane (Fig. 6.3). When a pigment molecule absorbs a photon it becomes excited; that is, an electron is moved to a higher or more energetic outer orbital (Fig. 6.4), increasing the energy of the pigment molecule by approximately the energy of the photon. The molecule will remain in the excited state for only about 1 ns (1 ns [nanosecond] = 10^{-9} s). Relaxation occurs by the transfer of this excited state or exciton to an adjacent chlorophyll, by return to the ground state with the production of heat equivalent to the excitation energy, or the re-emission of a less energetic (longer wavelength) photon and some heat.

The probability that an excited light harvesting chlorophyll will return to ground level with the emission of a photon establishes [O]-level fluorescence (F_o), which is therefore dependent on excitation light flux and is also indicative of the number of excited chlorophylls in the sample.

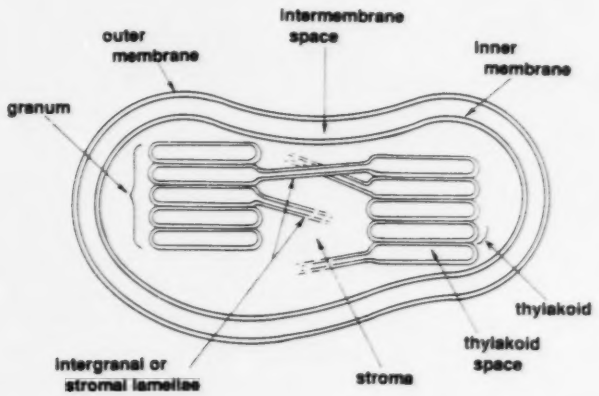


FIGURE 6.3. Schematic representation of a chloroplast, showing organization of the photosynthetic apparatus. Thylakoids are arranged in stacks called grana which are interconnected within the stroma by lamellae.

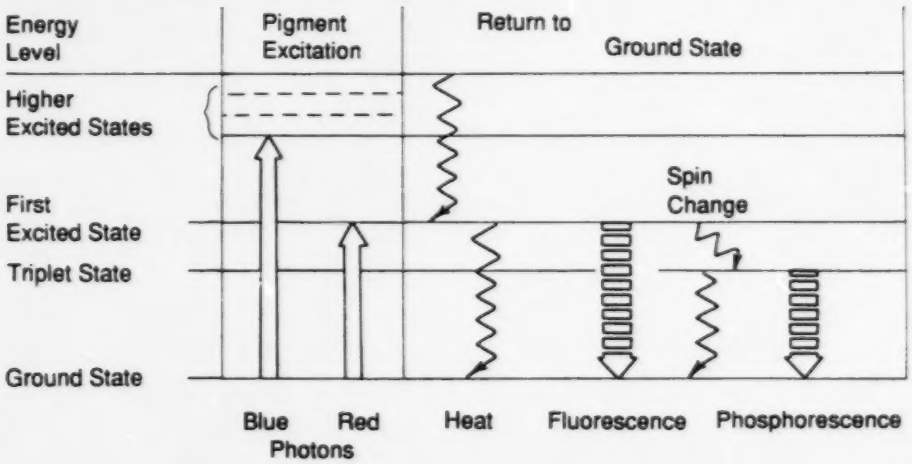


FIGURE 6.4. Diagram representing the energy levels of some of the main electronic states and transitions of chlorophyll. Straight arrows indicate light absorption; wavy arrows are radiation-less transitions to ground state with the release of heat; broken arrows are relaxations accompanied by the emission of a photon.

6.1.2 Energy transfer

The chlorophyll molecule that initially absorbed a photon can transfer this excitation energy or excited state to a neighbouring molecule. This process (exciton transfer) results in the relaxation of molecule one and excitation of molecule two, with no energy loss. There are about 300 antennae molecules associated with each PSII reaction centre (see Fig. 6.2) and, through a pattern of "random walk" transfers (up to 10^9 exciton transfers can occur in 1 s), the exciton can eventually reach and excite a reaction centre.

6.2 Photochemistry

6.2.1 Electron transfer to the primary acceptor

The PSII reaction centre is a complex of proteins, a chl_a molecule, and other components. It has been designated as P_{680}. In this environment, the excited chl_a can become oxidized by transfer of an electron to the primary acceptor (Q_A) (see Fig. 6.2). Thus,

$$P_{680} + h\nu \longrightarrow P_{680}^*$$
$$P_{680}^* + Q_A \longrightarrow P_{680}^+ + Q_A^-$$

This represents the first photochemical step of photosynthesis.

6.2.2 Reduction of Q_B and reoxidation of Q_A

The reduction of the secondary acceptor Q_B requires the transfer of two electrons from P_{680}:

$$P_{680}^* + Q_A \longrightarrow P_{680}^+ + Q_A^- \; ; \; Q_A^- + Q_B \longrightarrow Q_A + Q_B^-$$
$$P_{680}^* + Q_A \longrightarrow P_{680}^+ + Q_A^- \; ; \; Q_A^- + Q_B^- \longrightarrow Q_A + Q_B^{2-}$$

6.2.3 Reduction of plastoquinone and establishment of the transthylakoid proton gradient

The Q_B can be reoxidized by plastoquinone of which there is a large pool present in the thylakoid membrane:

$$Q_B^{2-} + 2H^+ + PQ \longrightarrow Q_B + PQH_2$$

The protons are accepted from the medium on the outside of the thylakoid and, when in turn PQH_2 is reoxidized by Fe-S/Cyt_f, these protons are released on the inside of the membrane (Fig. 6.2). This transmembrane transport of H^+ results in the establishment of a proton (pH) and electrical gradient across the membrane. This proton gradient represents the energy used in the production of ATP.

6.2.4 The oxygen evolving complex (OEC)

Electrons for the re-reduction of P_{680}^+ arise from the functioning of the OEC and its associated charge accumulating complex (Kok *et al.* 1970; Govindjee and Coleman 1990). The immediate electron donor on the oxidizing side of PSII is believed to be an amino acid and is designated Z:

$$Z + P_{680} + Q_A \xrightarrow{h\nu} Z + P_{680}^+ + Q_A^- \longrightarrow Z^+ + P_{680} + Q_A^-$$

Each OEC is associated with a highly ordered structure composed of specific polypeptides, chloride ions, and manganese atoms (Fig. 6.2). It is proposed that water oxidation proceeds through the photo-induced generation of four positive charges within the OEC:

$$S_0 \xrightarrow{h\nu} S_1^+ \xrightarrow{h\nu} S_2^{2+} \xrightarrow{h\nu} S_3^{3+} \xrightarrow{h\nu} S_4^{4+}$$

The equilibrium ratios of the dark pretreated system are believed to be:

S_0, 25%; S_1, 75%; S_2, 0%; S_3, 0%

S_4 is unstable and decays to S_0 with the release of an O_2 molecule. Protons from water oxidation are released into the intrathylakoid space and contribute to the establishment of the transthylakoid membrane gradient. Electrons are transported via the primary donor, Z, to reduce oxidized P_{680}. The existence of this proposed mechanism is supported by the fact that flashes of saturating light applied a few tenths of a second apart to plant cells or chloroplasts will induce no O_2 on flash 1, little or none on flash 2, a large amount on flash 3, and a reduced amount on flash 4 (Kok *et al.* 1970).

6.2.5 The intersystem electron transport chain

Once Fe-S/Cyt_f is reduced by electrons from the oxidation of PQH_2 (see Section 6.2.3 above), it in turn can be oxidized by plastocyanin (PC), the primary donor to the PSI reaction centre, P_{700}. Thus, the flow of electrons from PSII is via

$$Q_A \text{ ---> } Q_B \text{ ---> PQ ---> Fe-S/Cyt}_f \text{ ---> PC ---> to PSI}$$

6.2.6 Photophosphorylation

Photophosphorylation depends completely on electron transport (Fig. 6.2), but the electron transport rate itself depends to a large extent on coupling to phosphorylation. A partial explanation for this is that ATP is required for CO_2 assimilation and electron transport contributes to both an electrochemical and a proton gradient (Fig. 6.2) established across the thylakoid membrane (Avron 1981). Thus, photophosphorylation is regulated by the demand for ATP independently of the light energy absorbed by the thylakoid pigments, although the mechanism for this regulation is not well understood. It could be partially due to the uncoupling of outward proton flow from the phosphorylation action of ATPase (Avron 1981). If so, this represents a pathway by which excess energy may be dissipated. However, Stitt (1986) has suggested that sucrose synthesis ultimately controls the electron transport rate by regulating the recycling of inorganic phosphate.

6.2.7 Electron transport in PSI

The exact nature of the PSI reaction centre is uncertain. It may contain a chl_a in a special environment which is closely associated with a complex of Fe-S units. Once the primary acceptor (sometimes referred to as P_{430}) is reduced, it may be reoxidized by one of three pathways:

1. Via ferredoxin NADP reductase for the reduction of NADP

$$NADP^+ + 2H^+ + 2e^- \xrightarrow{\text{reductase}} NADPH^+ + H^+$$

2. Via Cyt_{b563} and Cyt_{b6} for cyclic electron flow.
3. Via the Mehler reaction which reduces molecular oxygen, forming the superoxide anion (Elstner 1982).

$$O_2 + e^- \text{ ---> } O_2^-$$

The $NADPH^+$ formed is used in CO_2 assimilation. Cyclic flow of electrons contributes to the transthylakoid H^+ gradient and is the reason that three ATP's can be produced per two $NADPH^+$'s. Oxygen competes with the other two pathways for electrons, and thus superoxide formation is inversely dependent on the demand for $NADPH^+$. Low CO_2 availability or blockage of biochemical CO_2 assimilation due to stress or low temperatures will therefore lead to the production of toxic oxygen species (see Section 7), which can be the cause of photodamage. Under conditions not normally conducive to efficient CO_2 assimilation, the Mehler reaction may serve to dissipate excess energy and therefore have a protective function.

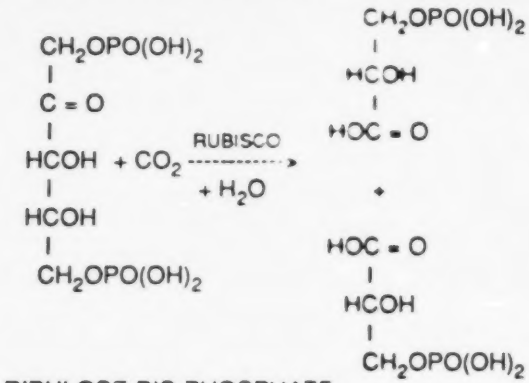
To summarize then, variable fluorescence emission (F_{var}) from PSII chlorophylls is an indicator of the redox state of the primary electron acceptor Q_A (Duysens and Sweers 1963; Papageorgiou 1975) (Fig. 6.2). It is believed by some investigators to be promoted by the charge recombination of the primary PSII electron donor acceptor pair, P_{680}^+/Q_A^- (see review by Amesz and van Gorkom [1978]; also Diner 1986; Klimov and Krasnovski 1981). At any point in time, F_{var} is a net measure of Q_A reduction from PSII

activity, minus the reoxidation rate on the PSI side of the electron transport system. Assuming the absence of intersystem quenching, any action resulting in Q_A reduction (e.g., OEC activity) raises the amplitude of F_{var}; its reoxidation (e.g., electron transport toward PSI) lowers the amplitude. When Q_A^- oxidation is blocked by the electron transport inhibitor, DCMU (dichlorophenyl, dimethyl urea), the fluorescence yield achieves a maximum (F_{max}) (Fig. 2.4). This maximum indicates the highest rate of P_{680}/Q_A^- charge recombination and suggests that OEC remains functional even though both O_2 evolution and intersystem electron transport to PSI are blocked by the inhibitor.

6.3 Biochemistry of CO_2 Assimilation

6.3.1 The photosynthetic carbon reduction (Calvin-Benson or C_3) cycle

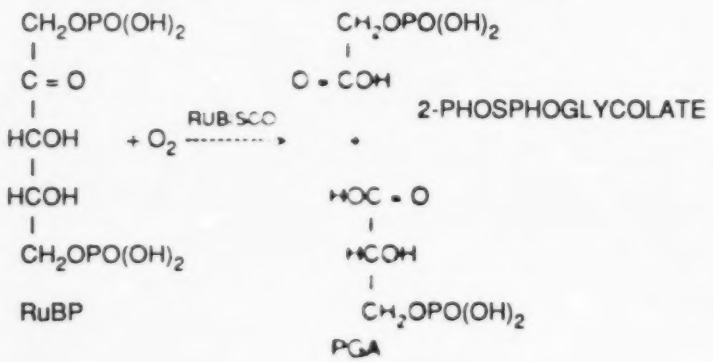
The photosynthetic carbon reduction cycle (PCR) is the predominant mechanism of CO_2 assimilation and represents the biochemical phase of photosynthesis. Because the energy stored in the carbohydrate end product is provided by photochemically produced ATP and $NADPH^+$, it is also a pathway for the conservation of excitation energy. The initial reaction is the CO_2 assimilation step catalyzed by the enzyme ribulose-bis-phosphate carboxylase (RUBISCO).



The rest of the cycle incorporates the energy of the high energy intermediates into carbohydrate and regenerates the RuBP from PGA (Bassham and Buchanan 1982). Six turns of the PCR are required to produce one hexose (a six-carbon sugar) molecule.

6.3.2 Photorespiration

The RUBISCO enzyme also acts as an oxygenase.



Oxygen and CO_2 compete for the enzyme and the production of glycolate rises as more O_2 is produced with increasing light intensity. Temperature is also a factor since O_2 solubility decreases more than that of CO_2 as temperature rises. This represents a case in which O_2 activity competes with energy-conserving processes. No CO_2 is incorporated in this reaction.

Two molecules of glycolate can be combined and with the release of a CO_2 molecule a three-carbon metabolite is regenerated. This process, which is called the photorespiratory pathway, requires the participation of enzymes present in three different cellular organelles: the chloroplast, the peroxisome, and the mitochondrion. Photorespiration represents one of several light-dependent pathways of energy dissipation and can account for the release of more than 30% of previously assimilated carbon (Nobel 1983).

6.3.3 The C_4 (Hatch-Slack) pathway

Some plant species (no conifers known so far) have a modified system for carbon dioxide uptake which, to a large extent, bypasses photorespiration. These plants, of which maize and sugar cane are examples, tend to be especially efficient at high temperatures and high light intensities. They are characterized as having the C_4 photosynthetic metabolic pathway (Lorimer 1981).

6.4 Summary of Photosynthetic Processes

Photosynthesis involves a large number of steps within the three phases of light harvesting, photochemistry, and biochemistry. While it is adaptive for plants to conserve as much of the absorbed light energy as possible, they must avoid the damaging effects of absorbing more energy than can be converted to photoproducts or safely dissipated by other means. Stress-resistant plants, including temperate conifers, manage this by having efficient mechanisms for eliminating toxic photoproducts, and very likely by being able to regulate photochemistry. This enables them to avoid the damaging effects of high light intensities during periods when environmental conditions are unfavourable for CO_2 assimilation. However, these mechanisms appear to demand the higher price of lower overall photosynthetic efficiency under favourable conditions than is paid by stress-sensitive plants. This difference permits the resistant plant to survive under rigorous winter or drought conditions and requires that the more efficient stress-sensitive plants complete their yearly growth or life cycles under either deciduous habit or as summer annuals.

The overall process of photosynthesis consists of energy-conserving and energy-dissipating steps. Energy conservation contributes to photosynthetic production, while dissipation detracts from it. It is when energy absorption exceeds the sum of conservation and dissipation that photodamage can be expected. The photoregulatory capacities of stress-resistant plants decrease the production of toxic photoproducts and increase the requirement for the dissipation of excess light energy. This requirement lowers photosynthetic energy conversion efficiency and proportionately increases the heat load on the needles or leaves of the resistant plant. The stress-resistant plant not only is able to accommodate this potential temperature rise, but it is able to regulate its activity in accordance with environmental variations and the season of the year.

The energy-conserving and energy-dissipating steps, as well as the potential for damage, are summarized below.

6.4.1 Energy-conserving steps

1. Light absorption by antennae pigments.
2. Exciton transfer to:
   - neighbouring chlorophylls
   - reaction centres
3. Photochemical production of the high energy nucleotide intermediates, ATP and $NADPH^+$.
4. Assimilation of CO_2 and carbohydrate (sugar) production (other possibilities exist, such as nitrate photoreduction, but are not considered here).

6.4.2 Energy-dissipating steps

1. Non-radiative decay of excited chlorophylls (heat production).
2. Triplet chlorophyll formation and carotenoid quenching.
3. Chlorophyll fluorescence.
4. Uncoupling of photophosphorylation from proton transport.
5. Back reactions of electron transfer.
6. The Mehler reaction: The production of superoxide anion and various other species resulting in the regeneration of H_2O and O_2.
7. Photorespiration.

The potential for photodamage exists when more light energy is being absorbed than can be dissipated through the sum of all the energy-conserving and energy-dissipating steps. By blocking the formation of toxic oxygen species, both photoinhibition and photoregulation contribute to protection from photodamage. (For more detail of these see Section 7.)

6.4.3 Destructive processes

1. Photo-oxidation of thylakoid pigments.
2. Peroxidations of thylakoid membrane lipids and other components because of the accumulation of toxic oxygen species.

Photodamage to chloroplasts may result from either of these destructive processes. Stress-sensitive plants have a limited capacity to resist their effects. On the other hand, stress-resistant plants must either minimize the adverse effects of photodamage or prevent its occurrence.

6.5 The Influence of the Components of Overall Photosynthesis on F_{var}

As a first approximation, in the photosynthesizing plant, anything resulting in an increased rate of Q_A reduction results in an increase in F_{var}. The response is therefore light-dependent, although the actual reduction rate is also determined by the extent to which PSII is subject to photoregulation. In addition, an increase in the reoxidation rate of Q_A diminishes the response. Therefore, at any light intensity the amplitude of F_{var} reflects the activities of both PSII and PSI. The activity of PSI also depends on both light intensity and the reoxidation rate of the primary PSI acceptor. This reoxidation is influenced by a wide variety of factors, such as the light energy available to PSI reaction centres, the availability of carbon dioxide, the effect of temperature on the biochemical reactions of CO_2 assimilation, the oxygen available for the Mehler reaction, the effect of temperature on the scavenging systems of toxic oxygen products, and changes in thylakoid membrane structure due to environmental factors.

Clearly, the activities of the two photochemical systems can vary independently from each other. Because of the number of factors which induce this variability, the yield of fluorescence emission is not—and cannot become—an indicator of photosynthetic efficiency or rates of photosynthate production.

To be useful, F_{var} assessment requires that the researcher or operator have some familiarity with the response patterns of particular plant types, measured systematically, as these plants experience environmental change at different seasons of the year. Such assessments necessarily involve a degree of subjectivity. However, users of the integrating fluorometer system will be rewarded with increased understanding of how nursery operations can be directed toward improved seedling quality.

7 PHYSIOLOGICAL BASIS OF ENVIRONMENTAL EFFECTS ON PHOTOSYNTHESIS

In Section 6, it was shown that the F_{var} response measured by the integrating fluorometer is an indicator of the level of the photosynthetic activity a plant is capable of at the time of measurement. In stress-resistant plants, such as conifer seedlings, photosynthetic photochemical activity is regulated in response to environmental variables. The function of this regulation is to protect the plant from the adverse effects of absorbing more light energy than can be used or dissipated. Stress-sensitive plants, such as many summer annuals, have little control over photochemistry and are easily photoinhibited, damaged, or even killed by high light intensity, especially when under water or chilling stresses.

To a large extent, the cause of photodamage is the toxic products formed when the rate of photochemistry exceeds the rate of photosynthetic carbon assimilation. There are three main ways by which plant photosynthetic photochemistry can be affected by light: photoinhibition, photodamage, and photoregulation. As each plant species is uniquely adapted to its environment, F_{var} responses can differ greatly in plant species and even in different provenances of the same species.

7.1 Photoinhibition

Since it is not due to gross changes in pigment concentration (Powles 1984) or to chloroplast membrane integrity (Kyle 1987; Critchley 1988), photoinhibition differs from photodamage. It results in a reduction of the photosynthetic capacity of a plant under conditions of light fluxes in excess of the requirement for photosynthesis (Öquist and Malmberg 1989), and it is independent of stomatal limitation of carbon dioxide diffusion. A partial or full recovery of photosynthetic activity is usually observed within minutes to hours after return to noninhibitory light conditions (Krause 1988). Photoinhibition may be adaptive in that it can restrict the formation of toxic oxygen species (see Section 7.2.2) in plants subjected to environmental stresses.

The following mechanisms have been proposed to account, at least in part, for observed photoinhibition.

7.1.1 Inactivation of electron transport

Photoinhibition may be due primarily to inactivation of the electron transport system in the thylakoids. The dominant effect seems to be an alteration of the reaction centres of PSII, leading to a decrease in primary photochemical efficiency (Anderson 1986). Photoinhibited thylakoids show an impaired electron transport from P_{680} to Q_A (see Fig. 6.2) (Krause 1988). Critchley (1988) concludes that photoinhibition is largely a result of modification of the functionality of the radical pair: the reaction-centre chlorophyll P_{680} and pheophytin of PSII.

7.1.2 Photoinhibition and the Q_B binding protein

According to Kyle (1987), the molecular change related to PSII photoinhibition is a dysfunction and degradation of the 32 kDa Q_B binding protein. Excess light may induce a structural change in the protein that renders it inactive for electron transfer reactions and susceptible to the action of a membrane-bound protease (Anderson 1986).

7.1.3 Shade-grown plants

Plants grown under low light levels are relatively more susceptible to photoinhibition than plants grown at high light intensities. Chloroplasts of shade-grown plants have increased thylakoid stacking (Davies *et al.* 1984) and a larger antenna pigment complement than high-light grown plants. The antenna size of PSII affects the extent of photoinhibition because it determines the rate of light absorption by PSII (Anderson 1986). High light-acclimated plant cells also have a faster repair capacity than shade-acclimated cells (Samuelsson *et al.* 1987).

7.1.4 Environmental stress

Photoinhibition may be increased by environmental stresses or initiated at lower light levels. Increased photoinhibition is observed at chilling temperatures and may result from decreased carbon assimulation or lowered protein repair rates (Wise and Naylor 1987b). Night frosts have also been shown to increase the susceptibility of Scots pine (*Pinus sylvestris* L.) to photoinhibition at high light levels the following day (Strand and Lundmark 1987).

7.1.5 Recovery from photoinhibition

Recovery from photoinhibition has been reported to involve *de novo* synthesis of Q_B protein (Ohad *et al.* 1984). High levels of mRNA for the Q_B protein are present in chloroplasts (Anderson 1986), where it is encoded for and synthesized. Repair of PSII by re-synthesis of Q_B protein occurs continuously in the light, and photoinhibition becomes evident only when the rate of Q_B protein inactivation or degradation exceeds the rate of repair or synthesis (Anderson 1986; Krause 1988).

7.2 Photodamage

7.2.1 Light and oxygen dependence

Particularly under conditions of environmental stress, prolonged exposure to high light levels can lead to photodamage (see review of Gillies and Vidaver 1990). Damage results from the production of toxic oxygen species which can inactivate reaction centres, cause the photodestruction of pigments (Anderson 1986), and lead to peroxidation of lipid membranes and proteins (Salin 1987). Photodamage is both light- and oxygen-dependent. Water deficits and low temperature stresses can increase the likelihood and amount of photodamage, often with lethal effect in stress-sensitive plants (Ben *et al.* 1987). The enzymes involved in carbon fixation are both temperature- and water stress-sensitive, but the photochemical reactions of photosynthesis are much less so (Levitt 1980). Therefore, under stress, excess absorbed energy may not be dissipated, even at moderate light levels. Photodamage is again, in this case, due to production of toxic or reactive oxygen species. Relatively stress-resistant plants, such as temperate evergreen conifers, appear to have mechanisms that protect them against photodamage while they are under stress conditions and during winter dormancy.

7.2.2 Reactive oxygen species

• SUPEROXIDE ($O_2^{\cdot-}$): Superoxide is produced by the Mehler reaction when O_2 competes for electrons on the reducing side of PSI ($O_2 + e^- \dashrightarrow O_2^{\cdot-}$) (Elstner 1982). It may also participate in an energy-dissipating mechanism since it allows continued electron flow through the photosystems in the absence of CO_2, thereby avoiding photoinhibition (Radmer and Kok 1976).

Superoxide can oxidize components of the photosynthetic system, such as sulphur compounds, ascorbic acid, cytochrome c, metal ions, and metal complexes. It has also been implicated in lipid peroxidation, membrane damage, cellular toxicity, and single-strand breaks in DNA (Fridovich 1986). It is not as toxic as other free radical species, but participates in reactions producing them (Elstner 1982).

• HYDROGEN PEROXIDE (H_2O_2): The most stable of the oxy-intermediates, hydrogen peroxide does not exhibit radical properties because its two outer orbitals are completely filled (Salin 1987). It can act both as an oxidant and mild reductant (Elstner 1982). It is formed by the dismutation of $O_2^{\cdot-}$ either spontaneously or via catalysis by the enzyme superoxide dismutase.

$$O_2^{\cdot-} + O_2^{\cdot-} + 2H^+ \dashrightarrow H_2O_2 + O_2$$

Hydrogen peroxide has also been suggested as an alternate substrate for the O_2-evolving complex in the dark (Frasch and Mei 1987). Johansen (1988) proposes that H_2O_2, rather than H_2O, acts as an electron donor in photosynthetic oxygen evolution in spinach. Because of the stability of H_2O_2 when acting as an electron donor, the O_2-evolving complex has to accumulate only two oxidizing equivalents to evolve one molecule of O_2 (Johansen 1988). This would be an advantage for light-limited plants, but has not been demonstrated in conifers.

Hydrogen peroxide does not react readily with organic substrates, but does tend to form complexes with transition metals. The major mode of H_2O_2 toxicity is through the oxidation of SH-groups (Salin 1987).

• HYDROXYL RADICAL ($OH\cdot$): The product of the univalent reduction of H_2O_2, the hydroxyl radical is formed via the Haber-Weiss reaction (Haber and Weiss 1934), which is catalyzed by a transition metal.

$$O_2^{\cdot-} + M^{n+} \longrightarrow O_2 + M^{(n-1)+}$$
$$H_2O_2 + M^{(n-1)+} \longrightarrow OH^- + OH\cdot + M^{n+}$$

Overall reaction:

$$O_2^{\cdot-} + H_2O_2 \longrightarrow OH^- + OH\cdot + O_2$$

The $OH\cdot$ is a strong oxidizing agent and reacts at nearly diffusion-limited rates with most organic compounds (Dorfman and Adams 1973). It reacts with the first available substrate: enzymes, small metabolites, nucleic acids, or membranes (Salin 1987).

• EXCITED SINGLET OXYGEN ($^1O_2$): Formed when one of the outer shell O_2 electrons is elevated to a higher orbital and its spin is inverted, singlet oxygen is an electronically excited species (Salin 1987). The resulting antiparallel spin is referred to as the singlet state. Singlet oxygen can be formed in a number of chemical, photochemical, and biochemical systems. The most common method of generation in chloroplasts is by the transfer of exciton energy from chlorophyll to dioxygen (Elstner 1982):

$$Chl \xrightarrow{h\nu} Chl^* \text{ (excited chlorophyll)}$$
$$Chl^* \longrightarrow {}^3Chl \text{ (triplet chlorophyll)}$$
$${}^3Chl + O_2 \longrightarrow {}^1O_2 + Chl$$

Singlet oxygen is highly reactive and can take part in addition reactions to enes and dienes, forming hydroperoxides and endoperoxides (Foote 1979). These propagate free radicals in chain-type reactions (Pryor 1976). It also can damage thylakoid membranes by oxidation of antenna chlorophyll molecules, resulting in pigment bleaching or peroxidation of the polyunsaturated hydrocarbon side chains of the membrane lipids (Elstner 1982; Wise and Naylor 1987a,b).

7.2.3 Protective mechanisms against photodamage

7.2.3.1 Need for protection

Ordinarily, neither stress-sensitive (i.e., herbaceous annuals) nor stress-resistant plants (e.g., a temperate conifer) are likely to undergo photodamage under environmental conditions conducive to the completion of their growth cycles. Both stress-sensitive and -resistant plants have effective mechanisms for the removal of toxic oxygen species. As environmental stress levels increase, the effectiveness of these protective mechanisms tends to decline. Photodamage to a shade-grown plant exposed to excessive light levels exemplifies how the capacities of both energy-dissipating pathways and the protective mechanisms can be overwhelmed.

7.2.3.2 Removal of toxic oxygen species

• SUPEROXIDE DISMUTASE: There are three classes of superoxide dismutase (SOD) isoenzymes in plant cells. The most abundant is associated with the chloroplast, but is also found in other organelles (Lumsden *et al.* 1977). A SOD catalyzes the reaction between two superoxide molecules according to the equation:

$$O_2^{\cdot-} + O_2^{\cdot-} + 2H^+ \longrightarrow H_2O_2 + O_2$$

These are highly active metalloenzymes with rate constants that approach the level of being diffusion limited (Fridovich 1986).

• CATALASES AND PEROXIDASES: In biological systems, catalases and peroxidases are responsible for the removal of H_2O_2 through the following analogous reactions:

$H_2O_2 + H_2O_2 \longrightarrow 2H_2O + O_2$ (catalase)

$H_2O_2 + R(HO)_2 \longrightarrow 2H_2O + RO_x$ (peroxidase)

Most catalase activity is associated with cytoplasmic peroxisomes (Halliwell 1982) as chloroplasts appear to be devoid of catalase. The H_2O_2 must diffuse from the chloroplast to these peroxisomes (Van Ginkel and Brown 1978), and removal of H_2O_2 from within chloroplasts depends partially on the abundant peroxidases which exhibit broad substrate specificity (Salin 1987).

• GLUTATHIONE PEROXIDASE/REDUCTASE SYSTEM: This system is important in the detoxification of oxyradicals and H_2O_2. Hydrogen peroxide is removed in a peroxidation of the thiol-containing tripeptide glutathione (GSH):

$2GSH + H_2O_2 \longrightarrow GSSG + 2H_2O$

Oxidized glutathione (GSSG) can then be converted back to the reduced form by a glutathione reductase:

$GSSG + NADPH^+ + H^+ \longrightarrow 2GSH + NADP^+$

Although no evidence has been found for a specific glutathione peroxidase in plant chloroplasts, it is possible that GSH is peroxidized by one or more of the plant peroxidases having broad substrate specificity (Salin 1987).

Glutathione appears to be the most abundant low molecular-weight thiol in higher plants, accounting for about 95% of the thiols in *Picea abies* L. needles (Rennenberg 1982), with a concentration from 0.1 to 0.7 mM (Koiké and Patterson 1988; Schupp and Rennenberg 1988). Between 47 and 69% of the GSH and the glutathione reductase was reported to be located in the pea (*Pisum sativa* L.) chloroplast (Klapheck *et al.* 1987).

In *Picea abies* needles, GSH content undergoes seasonal changes, with high concentrations in winter and early spring and low concentrations during the summer (Esterbauer and Grill 1978). It also displays a light-dependent but temperature-independent diurnal rhythm, with the highest concentrations occurring during mid-day and lower concentrations at night (Koiké and Patterson 1988; Schupp and Rennenberg 1988). In the summer, when the GSH concentration was low, the diurnal fluctuation in concentration exceeded 50% in *P. abies* needles (Schupp and Rennenberg 1988). Higher concentrations of GSH in spruce needles have been found in trees grown at high elevations where they are subject to greater stress, lower temperatures, and more intense light than those grown at low elevations (Grill *et al.* 1988). The response differences appeared to be related to the level of stress and not to provenance differences (Grill *et al.* 1988).

• ASCORBATE CYCLE: Ascorbate is present in *P. abies* chloroplasts at around 50 mM (Esterbauer *et al.* 1980). The ascorbate cycle also contributes to the removal of peroxide from the chloroplast. In this system, ascorbate is peroxidized and then rereduced (Elstner and Kramer 1973; Groden and Beck 1979). As well, GSH and $NADPH^+$ participate in this cycle:

Ascorbate + H_2O_2 → Dehydroascorbate + $2H_2O$

Dehydroascorbate + 2GSH → Ascorbate + GSSG

GSSG + $NADPH^+$ + H^+ → 2 GSH + $NADP^+$

A diurnal rhythm has been observed for ascorbate in spruce needles, with higher ascorbate levels occurring in the light than in dark. About 20% of the needles' total ascorbate is involved and fluctuates in response to light levels (Esterbauer *et al.* 1980). Castillo and Greppin (1988) found that exposure to ozone decreases the amount of reduced ascorbic acid and GSH as a result of direct interaction with free radicals. Dehydroascorbate reductase activity increased following exposure to ozone, and was a function of the ozone concentration, whereas glutathione reductase activity was not altered.

• ∝-TOCOPHEROL: Lipid peroxy radicals, $^1O_2$ and ^{3}chl are probably all quenched by ∝-tocopherol which is present within the thylakoid membrane (Foote *et al.* 1978). It can function as a "chain-breaking" antioxidant by trapping fatty acid acyl peroxyradicals formed during lipid peroxidation (Wise and Naylor 1987b). It can also reduce $O_2^{\cdot-}$ to H_2O_2, directly quench $^1O_2$, or become oxidized by it (Wise and Naylor 1987b). A single molecule of ∝-tocopherol can undergo approximately 100 $^1O_2$ quenching events before it is oxidized to ∝-tocopheroquinone (Foote *et al.* 1978).

Cellular and chloroplast levels of ∝-tocopherol, GSH, ascorbate, and NADPH$^+$ are likely linked through feedback mechanisms and the ∝-tocopheroquinone can be reduced again by ascorbate (Leung *et al.* 1981). These systems work together and are coordinated by the activities of many different enzymes. Enzymes controlling the oxygen-scavenging system have been shown to increase in activity under stressful environmental conditions (Smirnoff and Colombé 1988).

• CAROTENOID QUENCHING: Carotenoid pigments also protect against photooxidation by singlet oxygen. These $^1O_2$ quenchers are assumed to consist of a group of xanthophylls: zeaxanthin, antheraxanthin, and violaxanthins (Häder and Tevini 1987). These xanthophylls can be converted into each other through a process driven by photosynthetic electron transport called the xanthophyll cycle where,

zeaxanthin ⟷ violaxanthin ⟷ antheraxanthin

(Häder and Tevini 1987). Other carotenoids are also involved such as β-carotene which is also involved in quenching ^{3}chl.

^{3}chl + car —> chl + ^{3}car

^{3}Car decays to the ground state with the release of heat, thus dissipating excess light energy (Krinsky 1979; Krause 1988).

7.3 Photochemical Regulation

In being able to regulate photochemistry, resistant plants do not form excess amounts of toxic products. They therefore escape damage from excess light even under stress conditions. A decrease in the rate of photochemistry is reflected by a decrease in F_{var}, which is thus indicative of the plant's response to environmental factors. While the F_{var} response can also indicate damage to plant photosynthetic tissue, this does not normally occur in stress-resistant plants except under extreme conditions.

Reversible inactivation of PSII has been found in the desiccation-tolerant red algae *Porphyra sanjuanesis* Krishnamurthy (Sibbald and Vidaver 1987). Chlorophyll fluorescence assay has shown that this regulation may be present in some conifers (Hawkins and Lister 1985; Toivonen and Vidaver 1988; Vidaver *et al.* 1988a; Vidaver *et al.* 1989a,b). Their studies suggest that in white spruce and possibly other conifer species, photoregulation is under genetic control. Evidence for regulation has also been reported elsewhere. A diurnal regulation of water splitting in *Arbutus unedo* L. has been reported by Demmig-Adams *et al.* (1989), and Strand and Öquist (1988) found that the rate of oxygen evolution in Scots pine decreased following exposure to sub-freezing temperatures. Summer rates of ferricyanide-dependent oxygen evolution (Hill activity) in isolated class C chloroplasts of white spruce decreased during autumn by about 60%, remained low during the winter, and increased rapidly again in early spring (Senser and Beck 1977). A reversible decline in OEC activity appears to be a component of non-photochemical fluorescence quenching (Q_E) (Schreiber *et al.* 1986; Krause *et al.* 1988).

Restriction of oxygen evolution during winter, together with closed stomata, would decrease the amount of oxygen available to participate in unfavourable reactions. Consequently, lower concentrations of reactive oxygen species would be formed, lowering the risk of photodamage in the chloroplast.

Some authors designate any reversible inactivation of photochemical activity as photoinhibition. For example, Schreiber and Neubauer (1987) implicate a loss of PSII donor side activity, and Öquist and Malmberg (1989) suggest that in two pine species photoinhibition is due, at least in part, to impaired exciton transfer from PSII light-harvesting pigments to PSII reaction centres. We, however, consider that these examples are more properly representative of photoregulation, because they are not dependent on electron transport.

7.3.1 Daylength-dependent photoregulation

Seedlings of all British Columbia conifer species studied until now have shown a capacity for photoregulation (as considered above) when exposed to water deficits or temperature extremes (Vidaver *et al.* 1988a; Vidaver *et al.* 1989a,b). Seedlings of some species are also able to photoregulate in response to the season of the year (Vidaver *et al.* 1989a). For example, photochemical activity in white spruce seedlings, as indicated by F_{var} assay (see Fig. 5.4), begins to decline in mid- to late August. A similar decline in photosynthetic activity as assayed by CO_2 exchange begins somewhat later in September. By early November, apparent photosynthetic rates may approach zero (Table 5.2). This long-term inactivation appears to be relatively temperature independent, cued by decreasing daylength. It parallels bud set, the acquisition of cold hardiness, and the progression toward dormancy as described by Lavender (1985). Possession of this capacity would appear to be adaptive in preventing photodamage in a species such as white spruce, which grows in regions characterized by relatively short growing seasons followed by the sudden onset of cold rigorous winters. Monitoring F_{var} during the period of photochemical inactivation can assist, therefore, in the determination of lifting dates for this species. Once photochemical inactivation is achieved, the seedlings must meet their chilling requirement below 5°C (Vidaver *et al.* 1989a) for reactivation to the levels of the previous growing season to take place. Long-term cued inactivation has not been observed in coastal Douglas-fir or lodgepole pine.

7.3.2 Advantages and "cost" of photoregulation

A decline in F_{var}, while indicative of decreased photochemical activity, does not necessarily arise from any impairment of, or damage to, chloroplasts. Only in the case of irreversible photodamage should it be assumed that loss of variable chlorophyll emission represents other than adaptive functions serving to protect chloroplast structures from the effects of excess light. Both photoregulation and photoinhibition can decrease the formation of toxic photoproducts.

Photoregulation does this by limiting the production of molecular oxygen necessary to produce oxygen radicals, and it is therefore less dependent on the endogenous scavenging systems which can themselves be adversely affected by low temperatures or other forms of stress. In some healthy conifers, such as white spruce, a reversible decrease in F_{var} suggests that photoregulation is operative and that the possibility of photodamage during stress conditions has been minimized. Nevertheless, photoregulation does place an additional load on other means of energy dissipation (e.g., heat) within the light-harvesting pigments. Most temperate conifers, however, should be highly efficient in dissipating excess absorbed light energy as heat, and the main consequence would be a moderate increase in needle temperature (Larcher 1983). Even a 10°C rise in needle temperature at sub-freezing air temperatures would have little, if any, detrimental effect.

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